

**BRUNO HENRIQUE DE MORAES E SOUZA**

**Gastropod fauna on organic falls at the Southwest Atlantic deep-sea**

**Dissertation submitted to the Oceanographic  
Institute of the University of São Paulo, as partial  
fulfillment of the requirements for the degree of  
Master of Science, Oceanography program in  
Biological Oceanography**

**Advisor: Dr. Paulo Yukio Gomes Sumida**

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**University of São Paulo  
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**Original version**

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## ABSTRACT

The present study characterized the deep-sea gastropods community collected on whale bones and wood parcels artificially implanted in the deep Southwest Atlantic Ocean at 1500 and 3300 m depth for 23 months. A total of 5493 gastropods were collected and their distribution, abundance and diversity on substrates, depths and latitudes were calculated. Species richness and abundance of gastropods were higher in whale bones and deeper sites. Latitude did not seem to influence the distribution of mollusks. Five species were selected due to their abundance and possible ecological importance for further studies. Three were new species of the superfamily Abysochrysoidea and were morphologically and genetically described (two *Rubyspira* and one *Cordesia*). The other two were *Hyslogyrina rissoela* (Heterobranchia) and *Lusitanops cingulatus* (Neogastropoda). To understand their feeding behavior and dispersal strategies were conducted stable isotopes analyzes, observations of gut content, and radular and larval shell morphology. Larval shell of most species suggested planktotrophic development. Animals on different growth stages were found. Gastropods presented a diversity of feeding strategies, such as bacterial mats grazing, predation and specialized bone eating. The overlap of this fauna with other chemosynthetic environments and with other basins corroborate with the ecological stepping stone hypothesis. Besides that, phylogenetic studies about Abysochrysoidea were made. Their phylogenetic position within the Caenogastropoda are still uncertain but this group is considered closely related with Littorinidae. These snails are endemic from chemosynthetic environments, such as deep-sea hydrothermal vents, cold seeps and organic falls in the deep-sea. The genus *Rubyspira* is not settled at family level and no molecular studies were made for *Cordesia*. The present study also attempts to assign these groups phylogenetically. Concatenated COI, 16S, 18S and 28S and individual gene trees were constructed for maximum likelihood and Bayesian analysis. In all analyses the family Newtoniellidae were a sister group of Abysochrysoidea. Morphological and genetic evidences suggest that *Rubyspira* and *Cordesia* are closer related to *Abysochrysos* than to other provannid snails. The most accepted hypothesis is that Provannidae is paraphyletic. However, there are other ideas that indicates *Rubyspira* and *Cordesia* could be included in Abysochrysoidea or the Provannidae should be considered Abysochrysoidea. A morphologic revision of the superfamily should be made to better understand the relation within the group. Clades within the Abysochrysoidea were always well supported and similar to those found in the literature. The position of *Rubyspira* and *Cordesia* within Abysochrysoidea suggest whale bones and wood parcels did not play an evolutionary role as stepping stones for this group.

**Keywords:** Deep-sea. Whale falls. Wood falls. "Stepping stones". Abysochrysoidea. *Rubyspira*. *Cordesia*. Biodiversity. Deep-sea gastropods. Phylogeny.

## RESUMO

O presente estudo caracterizou a comunidade de gastrópodes de mar profundo coletados em carcaças de baleia e parcelas de madeira artificialmente implantadas no Sudoeste do Oceano Atlântico profundo a 1500 e 3300 m de profundidade por 23 meses. Um total de 5493 gastrópodes foram coletados e sua distribuição, abundância e biodiversidade nos substratos, profundidades e latitudes foram calculados. A riqueza e abundância dos gastrópodes foi maior nos ossos de baleia e em maiores profundidades. A latitude parece não ter influenciado na distribuição dos moluscos. Cinco espécies foram selecionadas devido à abundância e possível importância ecológica. Três são novas espécies da superfamília Abysochrysoidea que foram descritas morfolologicamente e filogeneticamente (duas *Rubyspira* e uma *Cordesia*). As outras duas foram *Hyalogirina rissoela* (Heterobranchia) e *Lusitanops cingulatus* (Neogastropoda). Para compreender os hábitos alimentares e de dispersão desses animais análises de isótopos estáveis, observações do conteúdo do trato digestório, da morfologia das rádulas e das conchas larvais foram realizadas. A concha larval da maioria das espécies sugere um desenvolvimento planctotrófico. Os moluscos encontrados apresentaram diversos tipos de hábitos alimentares como raspadores de carpete bacteriano, predadores e especialistas de ossos. A sobreposição dessa fauna com a de outros ambientes quimiossintéticos e de outras bacias oceânicas colabora com a hipótese ecológica das “stepping stones” no mar profundo. Além disso, estudos sobre a posição filogenética dos Abysochrysoidea foi feita. Sua posição nos Caenogastropoda ainda é incerta, mas são considerados próximos aos Littorinidae. Esses animais são endêmicos de ambientes quimiossintéticos, como fontes hidrotermais de mar profundo, fontes frias e quedas orgânicas. O gênero *Rubyspira* não está atribuído à nenhuma Família e não foram realizados estudos moleculares para *Cordesia*. Esse estudo também tenta organizar esses grupos filogeneticamente. Árvores concatenadas e individuais dos genes COI, 16S, 18S e 28S foram construídas para máxima verossimilhança e análise bayesiana. Em todos resultados a família Newtoniellidae foi um grupo irmão dos Abysochrysoidea. Evidências morfológicas e genéticas para que sugerem que *Rubyspira* e *Cordesia* estão mais próximos dos *Abysochrysos* do que de outros provannídeos. A teoria mais aceita é de que os Provannidae são parafiléticos. Porém há outras ideias de que *Rubyspira* e *Cordesia* poderiam ser inclusos em Abysochrysoidea, ou que os Provannidae fossem considerados Abysochrysoidea. Uma revisão dessa superfamília deveria ser feita para entender as relações desse grupo. Os clados de Abysochrysoidea sempre foram bem suportados como na literatura. A posição de *Rubyspira* e *Cordesia* nos Abysochrysoidea aponta que os ossos de baleia e parcelas de madeira possam não terem servido como “stepping stones” evolutivas para esses gastrópodes.

**Palavras-chave:** Mar profundo. Carcaças de baleia. Quedas orgânicas. “Stepping stones”. Abysochrysoidea. *Rubyspira*. *Cordesia*. Biodiversidade. Gastrópodes de mar profundo. Filogenia

## CHAPTER 1

### 1. Deep-sea organic falls and their gastropod fauna

#### 1.1. INTRODUCTION

The deep sea is the largest environment on Earth, reaching approximately eleven thousand meters (Ramirez-Llodra et al., 2010; Danovaro et al., 2014). It is also one of the least explored areas of the planet, mostly because of the huge depths and enormous size, covering almost 70% of Earth's surface, making exploration very costly. This results in a considerable undersampling of deep-sea organisms (McClain & Hardy, 2010). Below the 1000 m depth there is no sunlight and only very small variations in temperature and salinity. Dissolved oxygen is abundant in most places and currents are slow. However, these characteristics are not limiting to organisms and the real factor presssing life is the shortage of food due to the lack of primary production in most places. Animals living in the deep depend mostly on organic matter from the euphotic zone, but some may benefit from chemosynthetic primary production on hydrothermal vents and cold seeps (Ramirez-Llodra et al., 2010). In these regions, biomass is exceptionally high compared to areas receiving meager resources.

Chemosynthesis is fueled by reduced compounds and it is not restricted to vents and seeps. Large organic falls such as whale carcasses may induce the appearance of a chemosynthetic biota in deep areas owing to the massive localized input of organic carbon. Similarly to what occurs in other chemosynthesis-based habitats, a suite composed of generalists and specialists organisms colonize these areas (Smith et al., 1989). Wood parcels may also congregate specialist feeding on the substrate through symbiosis (Turner, 1973). These unique environments are considered important for the evolutionary history of many animals that are derived from organisms from shallow waters or other deep-sea habitats. Besides, many species come from ancient lineages, having

diversified within vents and seeps even at familiar levels with long time endemicity (Van Dover et al., 2002; Smith and Baco, 2003; Smith et al., 2015).

Both wood and large carcasses that reach the ocean floor can last long periods in the environment. These organic inputs attract animals that can use the organic falls directly as food sources, like plant structures, flesh, blubber and bones that are lipid rich. Other organisms utilize the organic compounds at the enriched sediment and bacterial mats to get energy (Turner, 1973; Smith et al., 2015). The organic input leads to a complex and unique faunal community that changes with time and use of the carcass, creating up to four overlapping successional stages (Smith et al., 2002, 2015; Smith and Baco, 2003).

On whale falls, the ecological succession varies according with size, depth and the degree of decomposition of the carcass. The first stage is characterized by the presence of big scavenger animals (hagfishes, sharks) eating the soft tissues, and it is known as the “mobile-scavenger stage”. It can last from 4 months to almost 2 years according to depth and carcass size. It is estimated that the soft tissues of an adult blue whale at bathyal depths may last for 5 years on the sea floor. At the end of this stage it is supposed that more than 90% of soft tissues have already been consumed by necrophagous animals (Smith and Baco, 2003). There is an accumulation of a diverse heterotrophic fauna (crustaceans, gastropods and polychaeta worms) that feeds on the leftovers of the larger fauna around the carcass and colonizes the exposed bones. Species abundance and biomass is one of the largest ever registered in deep-sea environments, decreasing quickly away from the whale. This stage resembles conditions of eutrophic places and it is called the “enrichment-opportunist stage”, that can vary substantially in time scale (Smith and Baco, 2003).

Subsequently comes the "sulfophilic stage", in which bacteria colonizing bones emit sulfides during the degradation of lipids (Smith et al., 1989; Smith and Baco, 2003; Fujiwara et al., 2007; Amon et al., 2013). This enable the arrival of animals from higher trophic levels, that feed on these bacterial mats, bone lipids and even predators. It is at this stage that whale falls fauna resemble that of wood falls, cold seeps and hydrothermal vents. However, they also present some species that have never or have rarely been found in other habitats (Smith

and Baco, 2003; Sumida et al. 2016). Similarly to other stages, the duration of “sulfophilic stage” is dependent upon whale size, ranging from 2 to 51 years. During this stage larger carcasses support much more complex trophic structures (Smith and Baco, 2003).

The last phase is known as the "recifal stage", where all the organic matter was consumed, and the bones are used as hard substrate by suspensivore animals. This stage can last for decades (Smith and Baco, 2003).

Wood falls shows a similar pattern of degradation of whale carcasses when colonized by wood-boring bivalves (Bienhold et al., 2013). When these mollusks feed on wood they deposit detrital organic matter enriching the sediments around the organic fall. This activity may last years and may also create a sulfophilic stage (Bernardino et al., 2010; Bienhold et al. 2013).

It is estimated that more than 850.000 whale carcasses could be scattered on the sea floor around the globe, in various stages of decomposition (Smith and Baco, 2003). However, despite their abundance only 9 natural whale falls were studied and sampled *in situ* (Smith et al., 1989; Fujioka et al. 1993; Smith and Baco, 2003; Goffredi et al., 2004; Lundsten et al., 2010a; Amon et al., 2013; Smith et al., 2014; Sumida et al., 2016; Amon et al., 2016).

Discussions about possible intersections among the fauna found on hydrothermal vents, cold seeps and organic falls, also known as the "stepping stones" hypothesis, were first conceived by Smith et al. (1989) and reinforced over the years (Distel et al., 2000; Smith and Baco, 2003; Smith, 2015; Smith et al. 2017). These large food falls work as organic islands in the deep sea helping organisms to disperse along large distances, playing ecological and evolutionary roles. The specialization on the feeding resources and the interaction among the fauna lead to speciation and the appearance of evolutionary novelties, such as some bivalves and polychaeta worms that have chemosymbiotic bacteria (Smith et al., 1989; Distel et al., 2000; Smith and Baco, 2003; Fujiwara et al., 2007; Lundsten et al., 2010). At higher taxa level, such as genus and family, connections between these faunas can be recognized in many cases, such as the gastropod *Provanna* (Warén and Bouchet, 2001; Braby et al., 2007) and species

of Neolepetopsidae (McLean, 2008). However, opposing ideas suggest organic falls are an independent evolutionary route with distinct biogeographic history, with only little input from vent fauna (Kiel, 2016, 2017). Kiel's idea was contested by Smith et al. (2017) that still supports the initial hypothesis.

Gastropods are a group of organisms that are present on every deep-sea chemosynthetic environment, with many endemic groups. Until last decade about 100 genera and more than 200 species of gastropods were found only in hydrothermal vents and cold seeps of the Indo-Pacific, Indian and Atlantic Oceans, what made the gastropods most species-rich group of vent animals known to date (Debruyeres et al., 2006; Sasaki et al., 2010). However, vents and seeps have been much more studied than whale and wood falls (Sasaki et al., 2010).

Failures to recognize distinct lineages often entails in an underestimation of species diversity. This is caused by incomplete sampling of growth series and life stages, common for deep-sea species, resulting in the misidentification of phenotypically plastic morphotypes or species (Johnson et al., 2015). These distinct evolutionary lineages with common morphological traits, also called cryptic species, are frequent among invertebrate inhabiting chemosynthetic environments (Vrijenhoek, 2009).

Deep-sea gastropods present many morphologic adaptations. They also inhabit different deep-sea environments and have a variety of feeding behaviors, allowing some deep-sea gastropods groups to survive extinct events that affected shallow-water taxa during Late Cretaceous and Early Cenozoic eras, making its diversification viable (Little and Vrijenhoek, 2003).

The deep-sea gastropods present common features such as (1) simplification of shell shape, (2) well developed periostracum, and (3) are frequently damaged by corrosion. These traits are due to calcification and predation, since shell of calcium carbonate is energetically costlier to produce and more easily corroded in deep-sea and predation is less common at this depth (Sasaki et al., 2010). Many deep-sea gastropods have a reduced digestive tract in relative size on species depending on chemosymbiotic bacterias (Warén and

Bouchet 1993, 2001). And some gastropods can present unique characteristics, such as the "scaly-foot gastropod" (*Chrysomallon squamiferum*) a monotypic genus, it has black deposits of iron sulfide minerals on its sclerites, this skeletal material is unique in metazoans (Warén et al., 2003; Suzuki et al., 2006a; Chen et al., 2015).

These gastropods present a wide variety of feeding behavior, such as (1) detritus and bacterial mats grazers, (2) filter feedings, (3) predators, (4) scavengers, and (5) chemosymbiotic bacteria. All of them have adaptation to dwell on these food sources, and some can take advantage of more than one type of food source (Sasaki et al., 2010).

Larval development is also a target of discussion of deep sea gastropods. They can have benthic or planktonic development, and the latter can present planktotrophy and lecithotrophy behavior, however no cases can be stated as well studied in deep-sea gastropods (Sasaki et al., 2010). The larval development can be inferred by the morphology of a well preserved larval shell, through its sculptures, number of whorls and size (Jablosnki and Lutz, 1980; 1983).

It is likely that gastropods have been inhabiting chemosynthetic environments for a long time. Kaim et al. (2008b) discover an ancient community of provannid-like shells on a pleisosaurid carcass from Late Cretaceous, like the ones found on modern whale falls. These sea snails from Abysochrysoidea Superfamily, wicdate bacj to 131 to 145 million years before the present (Kaim et al., 2008a). This group is commonly found at deep-sea habitats and it is composed of families Abysochrysoidea (Tomlin, 1927), Provannidae (Warén and Ponder, 1991) and Hokkaidoconchidae, a fossil group (Kaim et al., 2008a).

Abysochrysoidea is monotypic and the *Abysochrysoidea* is mainly found on deep-sea sediments off Brazil, West and South Africa, Oman and Indonesia, presenting mainly carnivorous and parasitic behaviors (Houbrick, 1979; Bouchet, 1991; Warén and Bouchet, 2009). The family Provannidae comprises a recent and well-known group of gastropods from deep-sea hydrothermal vents, hydrocarbon seeps, food falls and serpentinite-hosted ecosystems (Bouchet and

Warén, 1991; Warén and Ponder, 1991; Warén and Bouchet, 1993; Warén and Bouchet, 2009; Johnson et al., 2015; Chen et al., 2016a, 2016b). It is formed by 5 genera, *Provanna* (Dall, 1918), *Alviniconcha* (Okutani and Ohta, 1988), *Ifremeria* (Bouchet and Warén, 1991), *Desbruyeresia* (Warén and Bouchet, 1993), and *Cordesia* (Warén and Bouchet, 2009).

Besides the abyssochrysoid sea snail *Rubyspira* has not yet being assigned to any family. It is a bone specialist and was described for whale carcasses off Monterey, California (Johnson et al., 2010). Many other gastropods species dwelling on organic falls were described, most of them are opportunistic mollusks relying on the input of food provided by these events. They were found both on natural whale falls (Smith et al., 1989; Fujioka et al. 1993; Smith and Baco, 2003; Goffredi et al., 2004; Lundsten et al., 2010a; Amon et al., 2013; Smith et al., 2014; Sumida et al., 2016; Amon et al., 2016) and on artificial implanted carcasses and wood plots (Smith and Baco, 2003; Fujiwara et al., 2007; Braby et al., 2007; Lundsten et al., 2010b; this study).

The present study analyzed a total of 14 gastropod species, which is the richest diversity of this group hitherto found on natural or implanted food falls. These gastropods were also very significant in terms of size (and biomass) in some of the samples, with some specimens of a new *Rubyspira* species reaching over 40 mm in length. The small *Hyalogyrina rissoela*, described from hydrocarbon seeps of the African coast, was the most abundant mollusk found in the present study, and it is known to graze on bacterial mats (Warén and Bouchet, 2009). Other species with different life habits, include *Lusitanops cingulatus*, a carnivorous Neogastropoda found on organic falls and on sediments (Bouchet and Warén, 1980; Sumida et al. 2016). All these biological and ecological traits make the understanding of gastropod fauna essential for better knowing organic falls and chemosynthetic habitats.

## 1.2. OBJECTIVES

Most of studies on organic falls, natural or implanted, occurred on the Northeast Pacific Ocean, where fauna and its patterns were more thoroughly analyzed (Smith et al., 1989; Smith and Baco, 2003; Goffredi et al., 2004; Smith, 2006; Lundsten et al., 2010a, b). However, faunal patterns of this basin may not be the same in other regions, and biogeographical, populational and evolutionary data are still wanting for most basins (Lundsten et al., 2010). Only few studies were carried out in other ocean basins, such as the Northwest Pacific (Fujiwara et al., 2007), Southern Ocean (Amon et al., 2013; Smith et al., 2014) and the Southwest Atlantic (Sumida et al., 2016).

This study is part of the BioSuOr project (Biodiversidade e conectividade de comunidades bêmicas em substratos orgânicos no Atlântico sudoeste profundo – FAPESP 2011/50185-1), that aimed to understand the community, its connectivity, ecological traits and biodiversity on implanted organic falls of the Southeast Atlantic and compare it with different oceanic basins (detailed below).

The first objective of this work was to identify and describe the composition of the gastropod epifauna collected during the BioSuOr project and compare it with other food falls and chemosynthetic environments, since they were abundant and speciose group found in all implanted substrates (i.e., whale bones, wood parcels and inert material). The gastropod fauna was analyzed according to composition, substrate, depth and latitude. The dispersal strategy (protoconch morphology) and feeding behaviors (gut content and stable isotopes) was analyzed for the most abundant species.

The second aim was to properly classify and assign the genera *Rubyspira* and *Cordesia* to a family, since for the former species it is undefined and for the latter, dubious. And to observe if the “stepping stones” evolutionary hypothesis applies for this group.

### 1.3. BioSuOr

The BioSuOr Project (Biodiversidade de comunidades bênticas em Substratos Orgânicos - ossos de baleia e parcelas de madeira - no Atlântico sudoeste profundo) aimed to study the benthic community on artificially sunken whale bones and wood parcels on the Southwest Atlantic Ocean deep sea. This approach using experimentally implanted substrate is important because it is a rare event to find a natural whale carcass in the deep sea. To date only nine of them were found, and most at the Northeast Pacific Ocean.

The BioSuOr project had three main objectives: (1) to characterize the populations and diversity of benthic species on whale falls and wood parcels and compare them with communities from other oceans basins; (2) to observe the degradation rates on both substrates at different depths considering the diversity and abundance of consumers; and (3) to understand its connectivity.

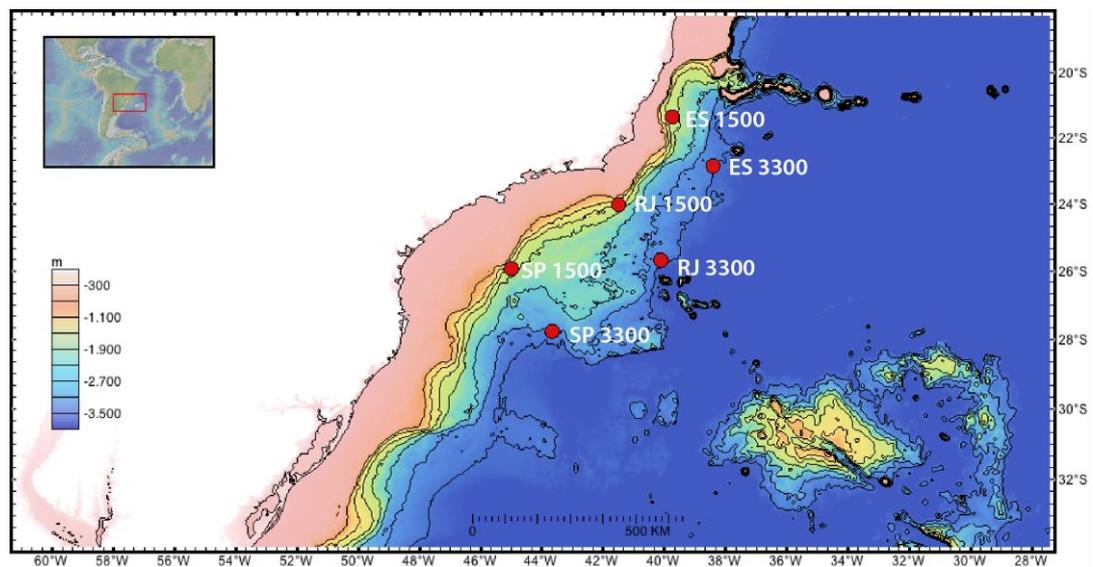


Figure 1.1. Bathymetric map of the studied region, with the sampling points location.

Organic plots were deployed along the Southeastern Brazilian continental margin on the 1500 m and 3300 m isobaths (Figure 1.1 and Table 1.1) using free-fall landers (detailed below). At 1500m the area is bathed by the North Atlantic Deep Water (NADW) characterized by salinities between 34,6-35, high levels of dissolved oxygen and temperatures ranging from 3°C to 4°C. At depth (3000m) the NADW mixes with the Antarctic Bottom Water (AABW) that

enters the Atlantic Ocean through the Drake Passage and can reach 30° S of latitude (De Madron and Weatherly, 1994).

Table 1.1. Coordinates and physicochemical traits of the points at moment were lander were dropped of BioSuOr project.

<b>Station BioSuOr</b>	<b>LATITUDE</b>	<b>LONGITUDE</b>	<b>Depth (m)</b>	<b>T °C (surface)</b>	<b>T °C (bottom)</b>	<b>Sal (surface)</b>	<b>Sal (bottom)</b>
ES-1500	-21.4501	-39.8965	1491	25.76	3.58	37.36	34.76
ES-3300	-22.8409	-38.4163	3322	25.11	2.42	37.15	34.9
RJ-1500	-24.0035	-41.5152	1413	25.44	3.52	37.36	34.62
RJ-3300	-25.3383	-39.6412	3285	23.74	2.45	36.92	34.91
SP-1500	-25.8940	-45.0348	1508	24.52	3.64	37.23	34.67
SP-3300	-28.0284	-43.5297	3358	22.94	1.03	36.82	34.75

Free-fall landers are independent experimental structures that have remained on the seabed to attract colonizing organisms. Structures were constructed in aluminum and equipped with three boxes containing whale vertebrae, three boxes with wood parcels and three boxes containing inorganic material for control. The position of each substrate in each box was randomly chosen. The boxes were open at their sides and contained a 500- $\mu$ m mesh, which allowed the exchange of water around the substrates. Each of these structures was equipped with deep-sea glass buoys and a Teledyne Benthos 866A acoustic releaser for recovery. The lids were kept open until they left the bottom upon retrieval (Figure 1.2).

Lander SP1500 was recovered earlier in October 2014, using the *R. V. Alpha Delphini* of the Instituto Oceanográfico da Universidade de São Paulo, after 16 months in the sea. The remaining structures were recovered in May 2015 with *N. Po. Almirante Maximiano* of the Brazilian Navy, remaining 23 months on the ocean floor. Only lander RJ1500 was not recovered because it could not be located during the second recovery cruise. In addition, the ES-3300 lander suffered damage after colliding with the propeller of the ship during recovery. Similar problems occurred with lander SP1500 which caused the loss of some samples.



Figure 1.2: Lander (experimental structure), boxes with mesh coating, with whale vertebra, control (inert material) and wood parcels (left to right). The acoustic release is in the center of the frame, keeping the box covers open. The buoys are the large yellow structure above the lander. Source: BioSuOr Material.

After recovery substrates were immersed in cold seawater (4°C) and photographed. The boxes containing the bones, wood and control were washed to remove the remaining epifauna. The macrofauna was removed from the substrates, separated into taxonomic groups, cataloged and preserved for taxonomy studies (4% buffered formalin) and molecular studies (95% ethanol).

## CHAPTER 2

### **2. Deep-sea gastropod diversity, habits and ecology in organic falls at the Southwest Atlantic**

#### **2.1. INTRODUCTION**

Mollusks are commonly found on chemosynthetic environments, and it is not different in organic falls such as whale carcasses and on sunken wood. These large organic inputs attract a suite of organisms, opportunistic and specialist that exploits this resource on different successional stages (Smith et al., 2002, 2015; Smith and Baco, 2003; Fujiwara et al., 2007; Lundsten et al., 2010b).

Gastropod is an important group in organic fall environments. Presenting different ecological roles related to its feeding strategies and they are present at almost all natural whale falls found (Smith et al., 1989; Fujioka et al. 1993; Smith and Baco, 2003; Lundsten et al., 2010a; Amon et al., 2013; Smith et al., 2014; Sumida et al., 2016; Amon et al., 2016). They have not been found in one natural whale falls discovered to date (Goffredi et al., 2004). And are recurrent as well in artificially implanted experiments (Smith and Baco, 2003; Fujiwara et al., 2007; Braby et al., 2007; Lundsten et al., 2010b; this study).

Mollusks inhabiting organic falls have reduced mobility, which implies that they must disperse to other carcasses as larvae in the water column. The morphology of their larval shells (protoconchs I and II) is an indicative of the type of larval development and it is preserved in the adult. Lecitotrophic juvenile gastropods have a yolk reserve that can nourish the individual until metamorphosis, while planktotrophic larvae feed on the plankton during its pelagic stage (Jablonski and Lutz, 1980).

The main differences in the larval shell of lecitotrophic and planktotrophic species are related to their sculpture, size and number of whorls. Lecitotrophic organisms have larval shells with smooth apices, reduced number

of whorls and almost no sculptures. Protoconch I is larger compared to protoconch II. On the other hand, planktotrophic gastropods have adorned larval shells, with many whorls and smaller embryonic shells (i.e. protoconch I). The whole larval shell is larger than those of lecitotrophic species (Jablosnki and Lutz, 1983).

The feeding strategies of gastropods are broad in all habitats, and it is not easy to specify food sources in food web, especially in the deep-sea. The least complicated and most direct form to investigate is to examine the gut and stomach contents, supplemented by stable isotopes analyses (Sasaki et al., 2010).

Several generalists or specialists species can be found, such as the sea snail *Rubyspira*, which consumes relatively large pieces of bones (Johnson et al., 2010). This genus was first described from the submarine canyon of Monterey, off California, on a whale carcass at 2893 m depth. Both species have symbiotic bacteria in their gills, but details of the symbiosis are still unknown. Both species also presented differences of feeding behavior, with one consuming the bone directly and the other, on the bone pieces lying on sediments surrounding the carcass (Johnson et al., 2010). The genus *Alviniconcha* and *Ifremeria* also present chemosymbiont bacteria, suggesting they rely part of their nutrition by this way (Bouchet and Warén, 1991; Warén and Bouchet, 1993).

Gastropods gather in large assemblages and commonly contribute to the general appearance of the biological chemosynthetic communities and on organic falls. They are often dominant, sometimes reaching more than 50% of collected epifauna (Bouchet and Warén, 1991; Smith et al., 2014; this study)

This fauna is from very ancient lineages, that may have survived extinction events during Late Cretaceous and Early Cenozoic eras, allowing its diversification (Little and Vrijenhoek, 2003). Fossils were found dwelling on large reptile carcass even before the emergence of modern whales (Kaim et al., 2008b).

The aim of this study is to identify and characterize the gastropod epifauna found in organic substrates implanted in the deep Southwest Atlantic

Ocean, patterns of distribution according with depth, latitude and substrate and possible faunal overlaps between basins and habitats were also verified. In addition, gut contents and larval dispersal strategies were examined for the dominant species. Three new Abysochrysoidea species are described, two of the genus *Rubyspira* and one *Cordesia*. *Rubyspira* species are whale bone specialists and the latter grazes on chemosynthetic microbial mats growing on wood parcels and bones. The gastropod fauna collected during the BioSuOr project is the richest ever found on food falls, being the dominant epifaunal group. Some species had their depth range of distribution increased.

## 2.2. MATERIAL AND METHODS

### Study sites, sampling and species choice:

All samples were collected during the BioSuOr project in October 2014 and May/June 2015, after 16 and 23 months left on the seafloor (respectively) on the Southeast Brazilian continental margin (Table 1.1.). Some specimens were preserved in molecular-grade ethanol for molecular genetic analyses and in 4% buffered formaldehyde for morphological identifications. All the remaining organisms were preserved in 70% ethanol for long-term storage.

Epifaunal gastropods were sorted according with morphology and counted. Five species were selected for further studies due their abundance and probable ecological importance: *Hyalogyrina rissoella*, *Rubyspira* sp. nov. 1, *Rubyspira* sp. nov. 2, *Cordesia* sp. nov., *Lusitanops cingulatus* (Appendix 2). Together they represented almost 97% of the gastropod fauna abundance.

Six species did not occur in enough numbers to allow a more detailed analysis (less than five individuals). They were identified to lowest possible taxonomic level: *Rubyspira* sp. 3, *Pseudococculinidae* sp., *Raphitomidae* sp. 2, *Raphitomidae* sp. 3, *Eulimidae* sp. and morphotype I. Other three species *Abyssochrysos* cf. *melanoides*, *Valvatoidea* sp. 1 and *Valvatoidea* sp. 2. and were separated for further studies, due to its abundance.

Morphological investigation and dissection were carried out on Zeiss SteREO Discovery. V8 stereomicroscope using individuals preserved in 70% ethanol or in buffered formalin. All specimens were photographed, and growth series plates were made for the five-main species. Shells morphometric measurements were made with Image J software (Image Processing and Analysis in Java).

The radulae of the main species were dissected and tissues around it were dissolved with 10% NaClO, washed in distilled water, and mounted on stubs with double-sided carbon tape and metallized with gold, using a Balzers SCD-050 sputter coater, and observed under a JEOL JSM- 5800LV scanning electron

microscope (SEM). SEM eletromicrographs were also made for adult individuals with detailed views of the embryonic and larval shells.

After the morphological identifications, DNA was extracted from the foot muscle, using the DNeasy Blood and Tissue Extraction Kit (QIAGEN) of at least three individuals of each main species.

The abyssochrysoid specimens were used for sequencing the mitochondrial cytochrome oxidase c subunit I (COI mtDNA) gene and the 16S ribosomal RNA (16SrRNA). Around 650 bp of COI were amplified by PCR using the primers HCO and LCO (Folmer et al., 1994), and around 550 bp of 16S rRNA with 16SAR and 16SBR primers (Palumbi, 1996). The PCR was carried out in a 12.5 µl reaction, including 2 µl of DNA template, 0.1 µl of both reverse and forward primers (20 µM), 6.25 µl Gotaq Mastermix dNTP mixture 2x, and 4.05µl sterilized deionized water. The thermocycling protocol was initialized at 95°C for 1 minute for denaturation, followed by 35 cycles of 45 seconds at 94°C, annealing at 50°C for 1 minute, an extension step at 72°C for 1 minute, ending with 72°C for 7 minutes. Then the amplification was confirmed with a 1.2% agarose gel electrophoresis. The purification was conducted with QIAGEN Purification Kit.

Bidirectional sequences were aligned and edited cheking its accuracy using the software Geneious (v11.0.3). Phylogenetic analyses and alignments were carried out using the resulting sequences and the available abyssochrysoid COI and 16S sequences available on GenBank, most dataset provided by Johnson et al. (2010), *Littorina littorea* (Caenogastropoda), *Neptunea antiqua* (after Johnson et al., 2010), and the lower heterobranch *Cornirostra pellucida*. The model was selected using JModelTest with BIC criteria. The Bayesian analyses were made in MrBayes software v3.2.6 for each locus individually and for the final concatenated phylogenetic tree.

The same procedures were applied for other species from different families, such as *Hyalogyrina rissoela*, (heterobranch) and *Lusitanops cingulatus*, (neogastropod). There were no hyalogyrinid species data on GenBank, so to insert it correctly in a phylogenetic tree, species of the same

superfamily (Valvatoidea) and other closer Heterobranchia were selected (see Dinapoli and Klussmann-Kolb, 2010), a Nudipleura (*Tomthompsonia antarctica*) and the Caenogastropoda *Littorina litorea* were selected as outgroups. For the Raphitomidae phylogeny, other species from its family were selected since it is a well-studied group and there is no genetic data of *Lusitanops cingulatus*. Here *Littorina litorea* was again selected as outgroup.

Dissection of the digestive system was made under a Nikon SMZ800 stereomicroscope with individuals stored in 70% ethanol. The gut content of 10 individuals of *Rubyspira* sp. nov. 1 and 10 *Cordesia* sp. nov. were removed, dried and put on a double-sided carbon tape for SEM on a JEOL JSM-5800LV scanning electron microscope.

Stable isotopes analyses of carbon and nitrogen, were carried out using foot muscle of ethanol preserved individuals of four of the main species *Rubyspira* sp. nov. 2 did not have sufficient numbers and was not analyzed. Tissues were frozen and freeze-dried for 24 hours, weighted and placed on tin capsules. At least three animals of each morphotype were selected for this procedure, with exception of *Hyalogyrina rissoela* for which two samples were pooled together to reach the minimum mass necessary for the analyses. Each organism was removed from different lander and substrates (i.e., whale bones, wood parcels and inert material) to check for differences in diet. Sample were sent to the School of Biological Sciences of Washington State University and analyzed with a Eurovector Elemental Analyser.

The results are expressed in delta notation,

$$\delta = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1\text{‰}],$$

where R is the ratio of heavy to light isotope, the standard referential in this study was atmospheric N<sub>2</sub> for nitrogen and Peedee Belemnite for carbon.

## 2.3. RESULTS

### Distribution, species richness and identification.

A total of 14 species of gastropods were found, of which five were better studied (i.e., morphology, genetics, nutrition and ecology) due to their abundance and probable ecological importance on organic falls (e.g., specialists) (Appendix 3). From the total of 5493 specimens *Hyalogyrina rissoella* (~69%) and *Rubyspira* sp. nov. 1 (~16%) were the most abundant species respectively (Figure 2.1).

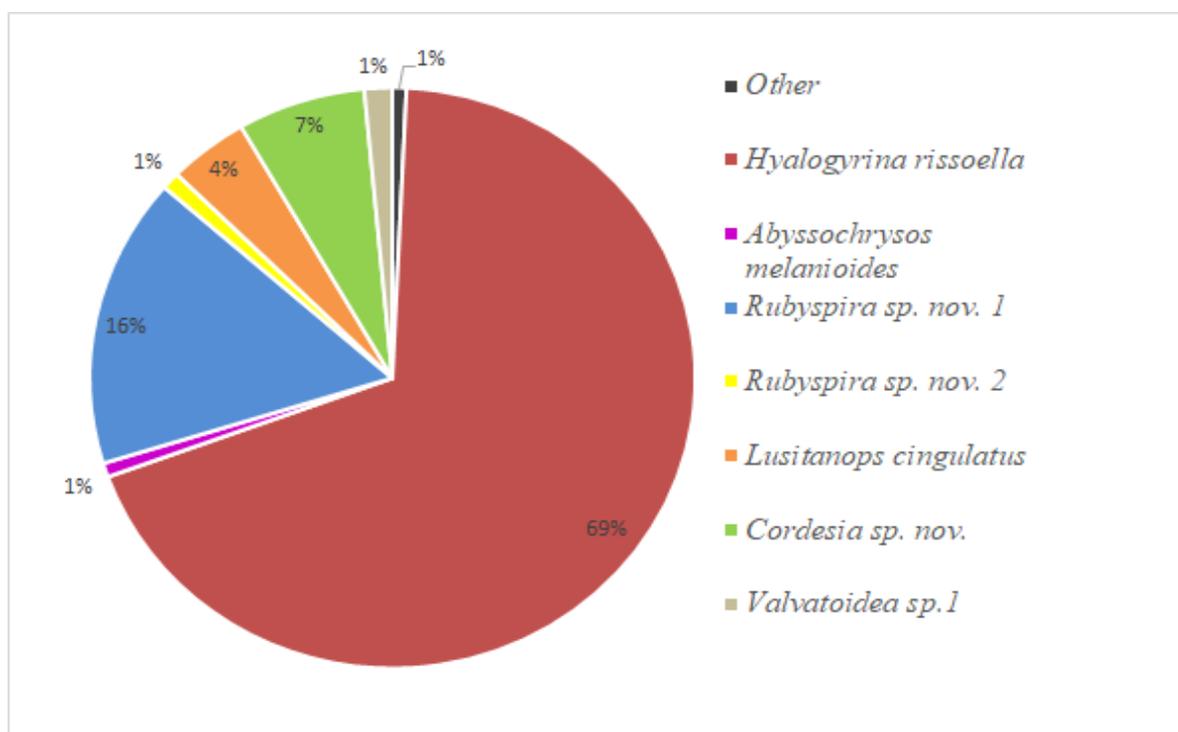


Figure 2.1: Relative abundance of each of the seven most abundant species (more than 40 specimens) to total of gastropods fauna.

Three new species were found and morphologically and genetically described in this study (Supplementary Material), two species of the genus *Rubyspira* (Johnson et al., 2010) and one of the genus *Cordesia* (Warén and Bouchet, 2009) (Figure 2.2). All species belong to Superfamily Abyssochrysoidea, of which two more species were identified, *Rubyspira* sp. 3 (see Sumida et al., 2016) and *Abyssochrysos* cf. *melanioides* (Tomlin, 1927) (Figure 2.3).

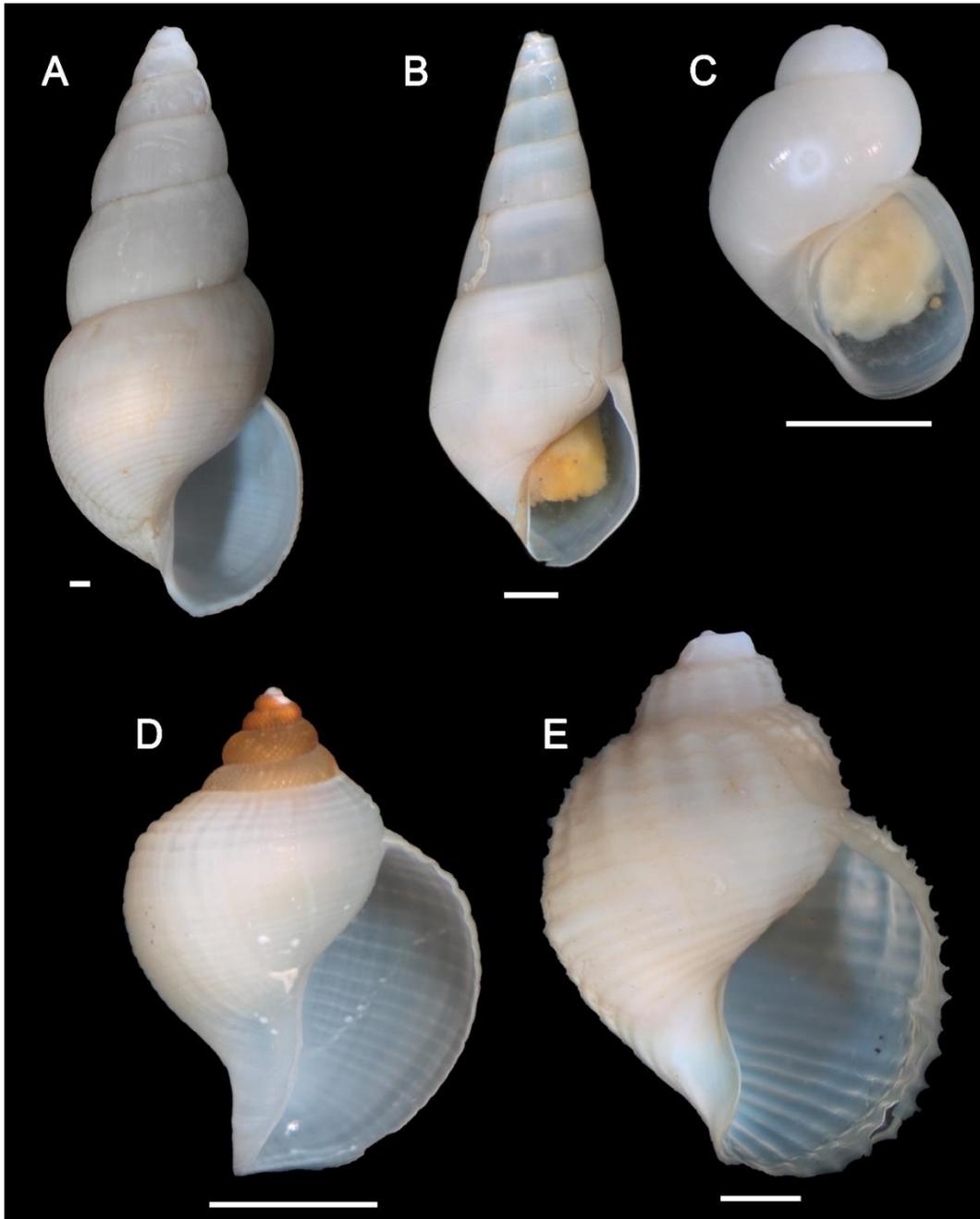


Figure 2.2: Stereomicroscope photography. The main species studied. A – *Rubyspira* sp. nov. 1, B – *Rubyspira* sp nov. 2, C – *Hyalogyrina rissoela*, D – *Lusitanops cingulatus*, E – *Cordesia* sp. nov. All individual scales bar represent 1000 $\mu$ m.

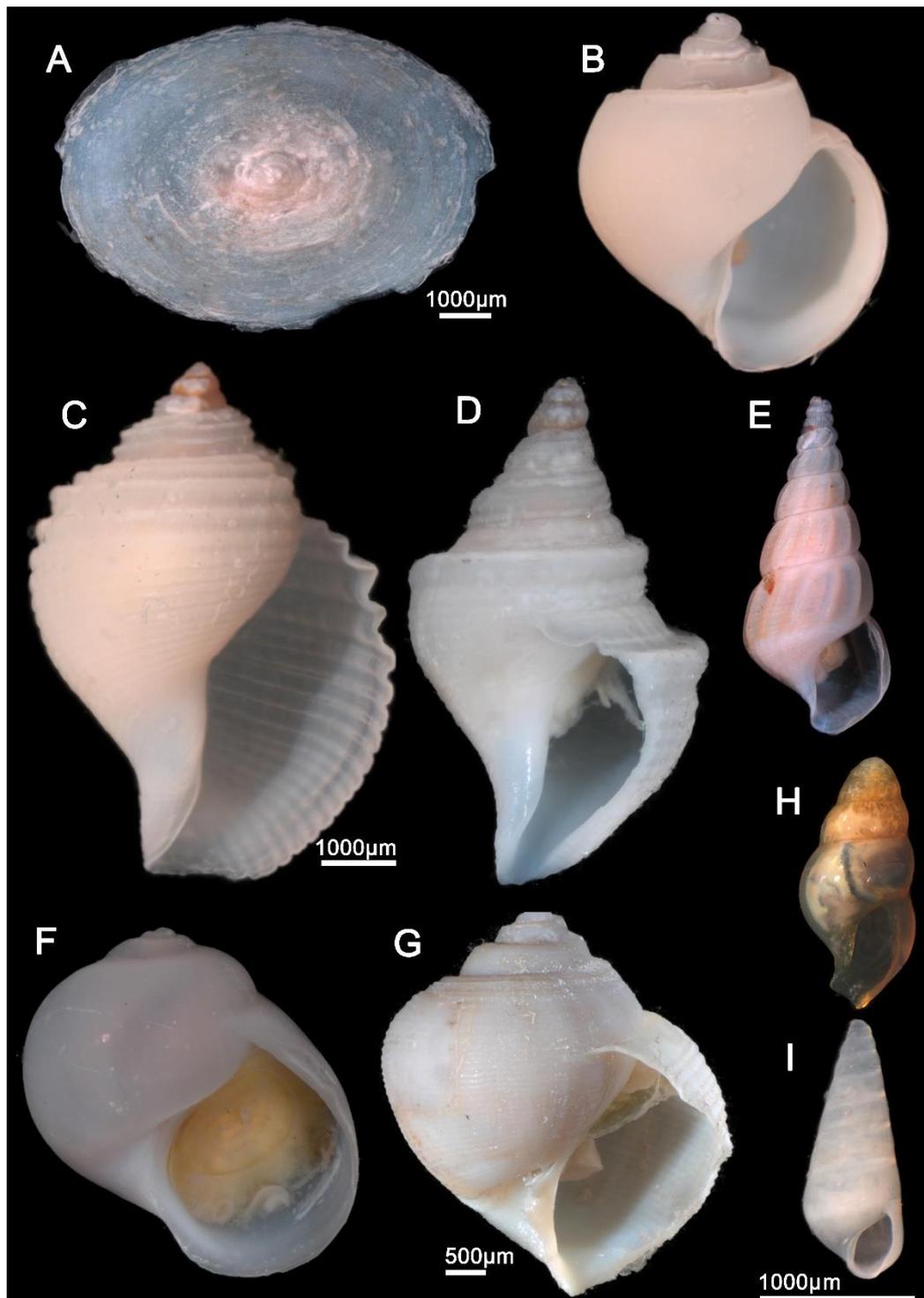


Figure 2.3: Stereomicroscope photography. A - *Pseudococculinidae* sp., B - *Valvatoidea* sp. 2, C - *Raphitomidae* sp. 3, D - *Raphitomidae* sp. 2, E - *Abyssochrysos* cf. *melanioides*, F - *Valvatoidea* sp. 1, G - *Rubyspira* sp. 3, H - Morphotype I, I - *Eulimidae* sp. 1. Central scale for C, D, E, F, H.

Neogastropods were represented by three species, all from family Raphitomidae (Bellardi, 1875). Two of them were rare species and less than five specimens were collected (Figure 2.3). The only one that was identified to species level was *Lusitanops cingulatus* (Bouchet and Warén, 1980) (Figure 2.2). Three species of Heterobranchia from Superfamily Valvatoidea were found, of which two species could not be identified (Figure 2.3) and the third was the most abundant species found in this study, *Hyalogyrina rissoela* (Warén and Bouchet, 2009) (Figure 2.2).

Eulimidae (Philippi, 1953) was represented by only one individual of an unidentified species, probably a parasite on echinoderms. Only two specimens of a limpet species were found, probably of the family *Pseudococculinidae* (Hickman, 1983). Only one gastropod specimen could not be identified (Morphotype I) (Figure 2.3).

Patterns of distribution could be observed among gastropods collected by the BioSuOr project. It seems that depth and type of substrate were the factors that influenced the most abundance and species richness at landers. There were no significant differences in species richness between latitudes. And the disparity in abundance can be explained because one of RJ landers was missed and one of ES was not properly rescued, so SP sites summed more specimens.

In terms of abundance lander SP-3300 had the highest number of individuals, mostly belonging to *Hyalogyrina*. Landers ES-1500, RJ-3300, ES-3300 and SP-1500 had decreasing numbers of gastropods. Lander RJ3300 was the richest with 10 morphotypes, followed by ES-1500 and ES-3300 with 8 species each, by SP3300 (7 species) and SP1500 (6 species). Lander SP-1500 remained almost 8 months less at the sea floor than other landers.

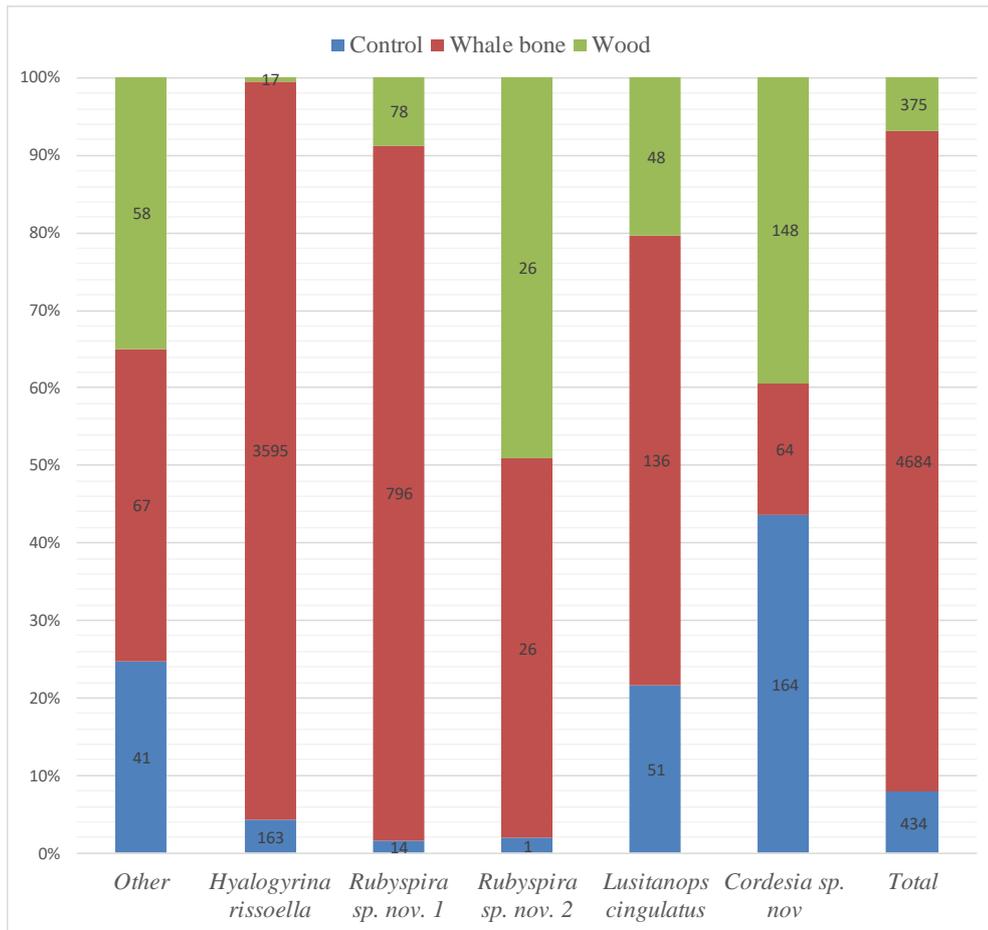


Figure 2.4: Distribution of the five gastropod main species among substrates of BioSuOr Project on the Southwest Atlantic Ocean. Numbers are equivalent to abundance of each species.

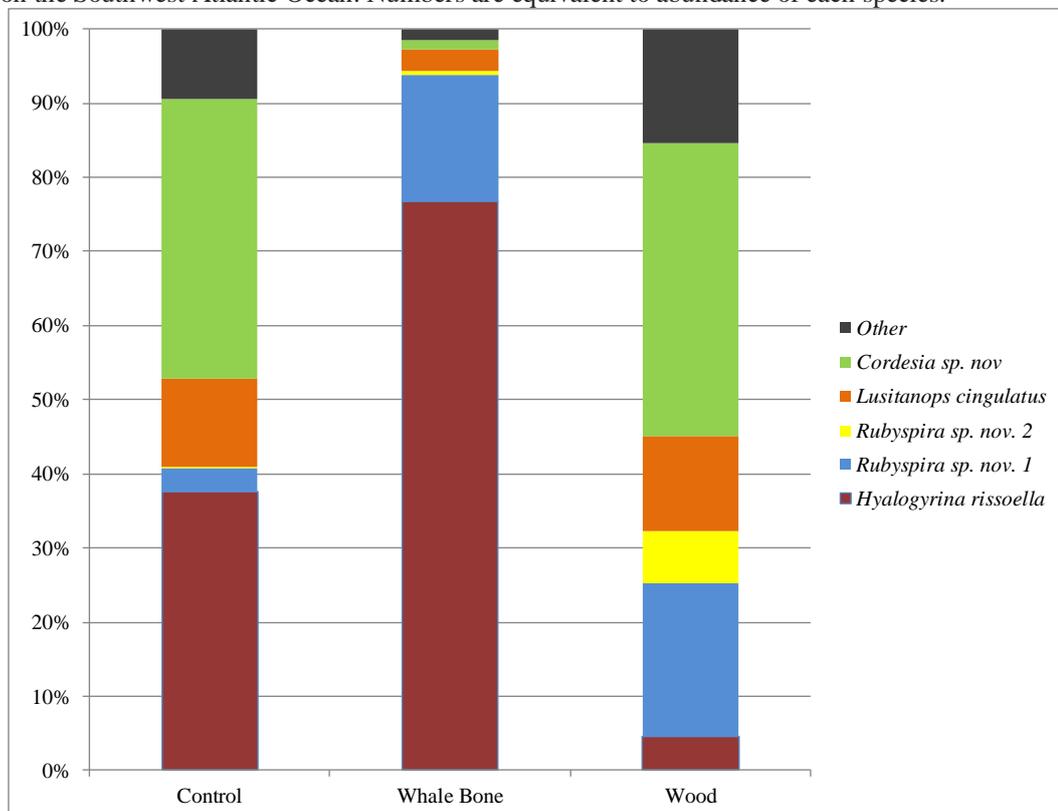


Figure 2.5: Species richness between substrates of BioSuOr Project on the Southwest Atlantic Ocean and composition of gastropod fauna.

Most of gastropods settled on whale bones, representing 85.3% of the total gastropod fauna, followed by inert material (control) with 7.9% and 6.8% on wood parcels (Figure 2.4). Both organic substrates had more species richness than the inert material. The richest substrate was the whale bone with 12 gastropod species, only *Pseudococculinidae* sp. and mollusk Morphotype I were absent, both are rare species with only two and one individuals respectively. Woods (11 species) and inert material (8 species) had smaller numbers (Figure 2.5).

Landers deployed at 3300 m depth exhibited higher gastropod abundance (71.6%) than shallower ones. All *Cordesia* sp. nov., *Rubyspira* sp. 3 and Valvatoidea sp. 2 individuals were found at 3300 m depth, while *Rubyspira* sp. nov. 2 was present only at 1500m landers. Most of other abundant species were found in both depths, such as *Rubyspira* sp. nov. 1, *Lusitanops cingulatus*, *Hyalogyrina rissoella*, *Abyssochrysos* cf. *melanioides* and Valvatoidea sp. 01 (Figure 2.6).

Species richness was also higher at 3300 m sites (12 species), where nearly all gastropods species were present except for Eulimidae sp. and *Rubyspira* sp. nov. 2, the former is a rare species (1 individual) and the second one seems to inhabit only shallower sites. Only nine species occurred in the 1500 m landers, excepted for *Cordesia* sp. nov., all more abundant gastropods were also found at these sites (Figure 2.7).

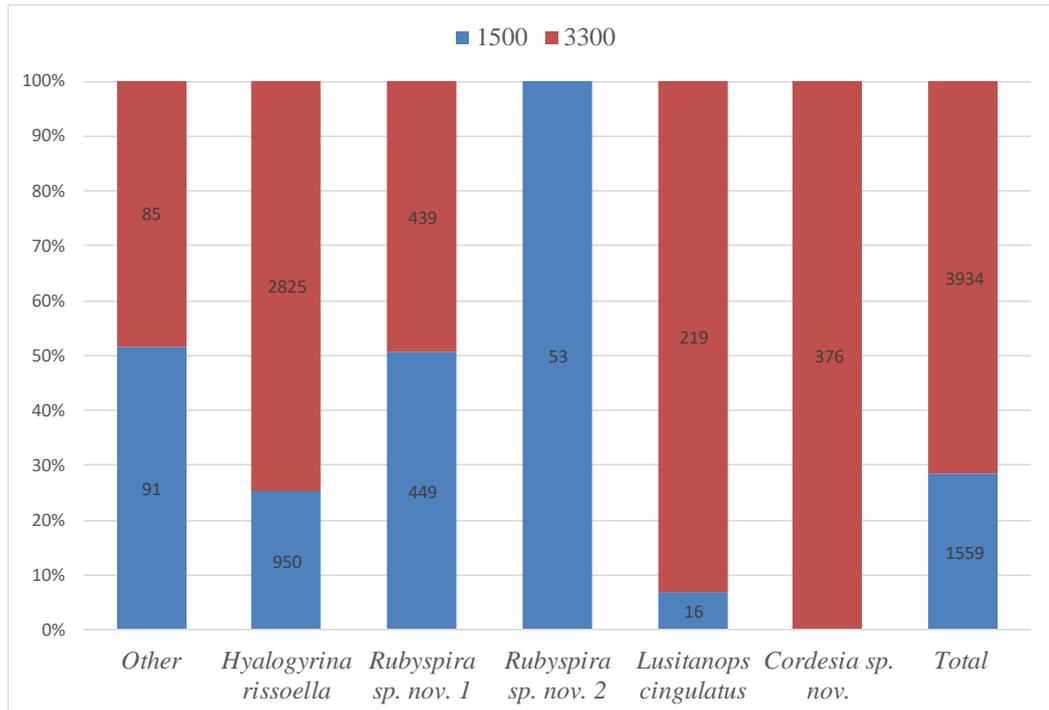


Figure 2.6: Distribution of the five gastropod main species among depths of BioSuOr Project on the Southwest Atlantic Ocean. Numbers are equivalent to abundance of each species.

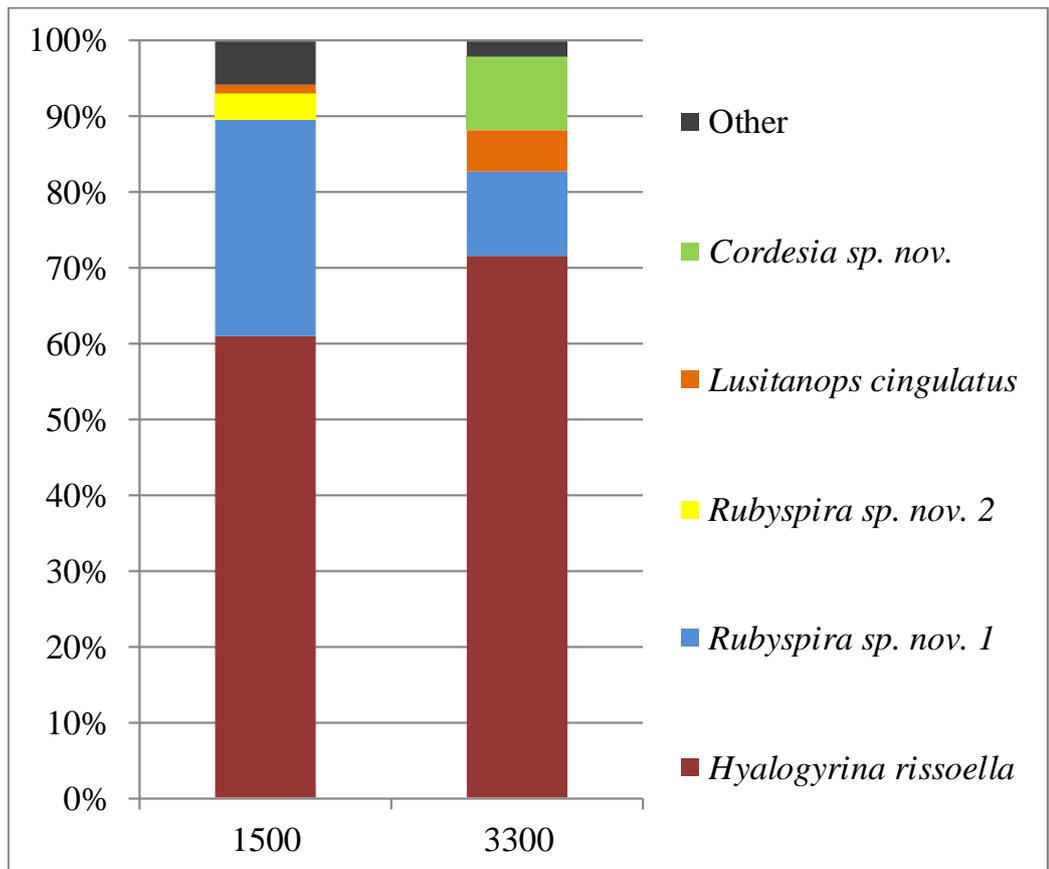


Figure 2.7: Species richness between depths of BioSuOr Project on the Southwest Atlantic Ocean and composition of gastropod fauna.

### **Gut content, size and mode of development.**

Juveniles of all main gastropod species were found on the substrates, as well as specimens of increasing size including supposedly fully-grown adults (Table 2.1).

The embryonic and larval shells of both new *Rubyspira* species, *Cordesia* sp. nov., and *Lusitanops cingulatus* were observed in detail. They all bear a reduced embryonic shell compared to the larval shell, well defined axial and spiral sculptures and high numbers of whorls, traces of planktotrophy. Analysis of the morphology of *Hyalogyrina rissoela* larval shell were not conclusive since there is no clear demarcation of the protoconch I. However, there are few whorls at the apex of the shell and no sculptures.

Stable isotopes analyze were conducted to support the direct observation of gut content of gastropod species and obtain stronger nutritional results (Table 2.2). The stable isotope of nitrogen presented high variations on whale bones, on wood parcels and bacterial mats, and  $\delta^{13}\text{C}$  were more variable on bones. The highest mean for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were both on bones, and the lowest on bacterial mats and woods respectively.

Consequently, most of individual values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were also very variable, some that were very low or high were treated as artifacts and removed from the analysis and consequently not used to calculate the mean or standard deviation of stable isotopes. *Cordesia* sp. nov. presented the highest values for  $\delta^{13}\text{C}$  and *Hyalogyrina rissoela* was the most depleted. The latter species also had the lowest mean value for  $\delta^{15}\text{N}$  and *Lusitanops cingulatus* was the most enriched organism in  $\delta^{15}\text{N}$  of this study. All standard deviations can be assumed as high, excepted for *Hyalogyrina rissoela*  $\delta^{13}\text{C}$ , and *Cordesia* sp. nov.  $\delta^{15}\text{N}$  (Table 2.2).

Table 2.1: Morphometric of study's five main species, with maximum and minimum individual's sizes.

Species	Maximum (mm)				Minimum (mm)			
	Shell Height	Shell Width	Opening Height	Opening Width	Shell Height	Shell Width	Opening Height	Opening Width
<i>Rubyspira</i> sp. nov. 1	23.3	9.0	8.5	5.1	1.3	0.8	0.7	0.4
<i>Rubyspira</i> sp. nov. 2	10.7	4.8	3.7	2.4	2.9	1.3	1.1	0.7
<i>Cordesia</i> sp. nov.	7.2	4.8	4.5	3.0	1.6	1.1	1.0	0.7
<i>Hyalogyrina</i> <i>rissoela</i>	2.6	1.6	1.4	1.2	0.8	0.7	0.6	0.4
<i>Lusitanops</i> <i>cingulatus</i>	10.8	6.7	7.1	3.8	1.7	0.8	0.8	0.3

Table 2.2: Mean and standard deviation (SD) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios (‰) of for of the main species of this study, and the main available sources of nutrition. Enrichments of 0-1‰ for  $^{15}\text{N}$  and 3%–4‰ for  $^{13}\text{C}$  are generally relative to diet.

Species	n	$\delta^{13}\text{C}$ (‰)	$^{13}\text{C}$ standard deviation	$\delta^{15}\text{N}$ (‰)	$^{15}\text{N}$ standard deviation
<i>Rubyspira</i> <i>sp. nov. 1</i>	4	-23.89	1.79	6.22	1.52
<i>Cordesia</i> <i>sp. nov.</i>	3	-23.12	1.83	5.64	0.46
<i>Hyalogyrina</i> <i>rissoela</i>	2	-27.72	0.22	4.76	1.61
<i>Lusitanops</i> <i>cingulatus</i>	3	-23.65	1.28	8.10	1.33
<i>whale bone</i>	6	-27.00	2.54	5.70	0.99
<i>wood plots</i>	5	-27.32	0.68	2.93	1.49
<i>bacterial mats</i>	6	-30.47	0.27	2.94	2.62

Mollusca Linnaeus, 1758;  
Gastropoda Cuvier, 1797;  
Caenogastropoda Cox, 1959;  
Abyssochrysoidea Tomlin, 1927;  
Unassigned Family;  
*Rubyspira* Johnson, 2010;  
***Rubyspira* sp. nov. 1**

*Rubyspira* sp. nov. 1 is a new Abyssochrysoidea species. This genus is not settled at family level due to unconformity between morphology and genetic studies. This species was morphologically and genetically described of the present study (Supplementary material). The adults of this gastropod resemble those of *Rubyspira osteovora* collected at Monterey Canyon, but they have a smoother spiral sculpture. Juveniles can be easily confused with *Rubyspira* sp. nov. 2 younglings, but *Rubyspira* sp. nov. 1 presents a slightly more ovate adult shell.

It was a very abundant species collected during the BioSuOr Project, the second most abundant gastropod, summing 888 specimens. It was also present on every lander but presented different stages of development. The larger specimens were found at RJ-3300, this supposed adult individuals were collected with many juveniles, it was also in this area they were most abundant and the dominant species. The same pattern was observed on ES-3300. On other sites were collected only smaller specimens, with only few exceptions.

As a specialist species, *Rubyspira* sp. nov. was collected mainly on whale bone (87.3%), and they are the dominant species on this substrate, probably also in terms of biomass since the larger individual reached more than 2cm. It is the main species that settled lesser in inert material (1.6%). Regarding depth, this species occurred almost evenly in shallower (ca. 50.6%) and deeper (49.4%) stations.

Their larval shell has planktotrophic traits such as reduced embryonic shell compared to the larval shell, well defined axial and spiral sculptures and large numbers of whorls (2.5 well defined whorls). The initial part of protoconch I is slightly opened and sealed with a calcareous structure due to autotomization,

resembling that of the provannids snails (Supplementary Material, Figure 2.11). Larval shell is commonly lost on fully grown individuals.

Its radula is taeniglossa, its marginal teeth apice are crowbar-like and the lateral teeth apice are hook-shaped. The strong and modified teeth seem to be used to facilitate the direct removal of bone (Supplementary Material, Figure 2.9).

The enrichments of 0-1‰ for  $^{13}\text{C}$  and 3%–4‰ for  $^{15}\text{N}$  are generally relative to diet were observed on stable isotopes analyzes when compared to whale bone results, suggesting they probably feed of this substrate. In addition, the direct observation of *Rubyspira* sp. nov. 1 gut content revealed large pieces of whale bone together with smaller fragments and sediment particles (Figure 2.8). Bones were found on every dissected specimen.

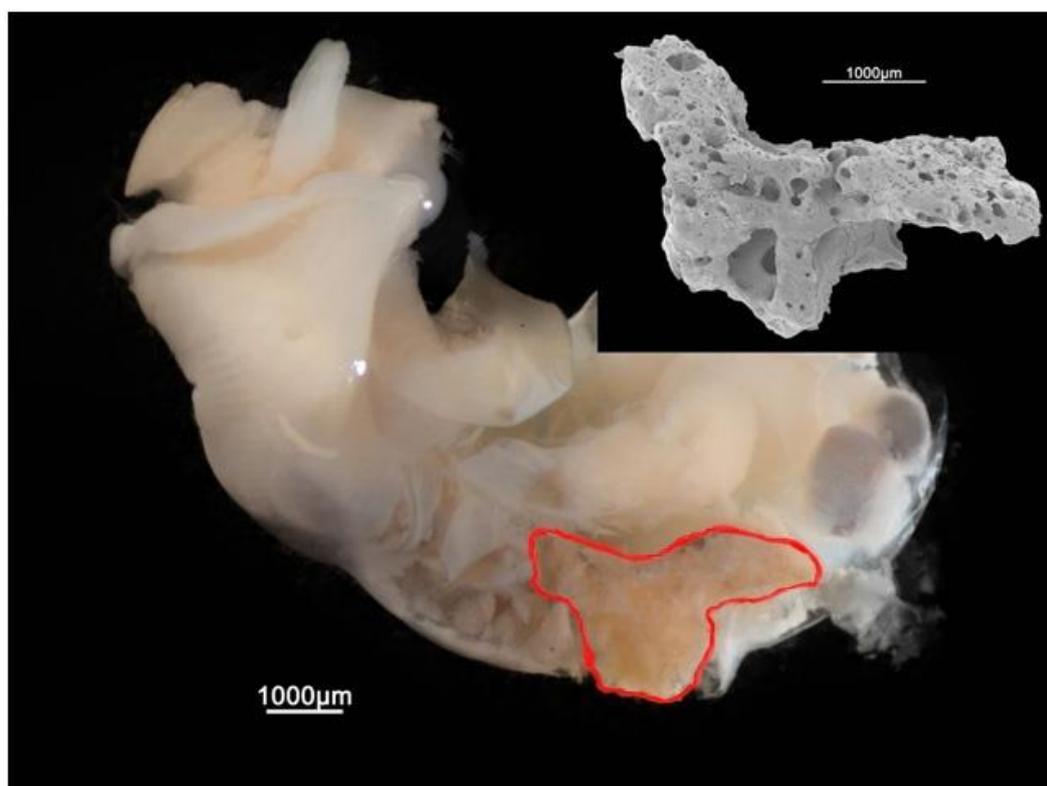


Figure 2.8: Photograph of *Rubyspira* sp. nov. 1 removed from its shell showing a large piece of whale bone within its dissected stomach (highlighted in red). A scanning electron micrograph of the same bone piece is shown on the top right

Mollusca Linnaeus, 1758;  
Gastropoda Cuvier, 1797;  
Caenogastropoda Cox, 1959;

Abyssochrysoidea Tomlin, 1927;  
Unassigned Family;  
*Rubyspira* Johnson, 2010;  
***Rubyspira* sp. nov. 2**

This *Rubyspira* is a new species and was also described morphologically and genetically on the present study (Supplementary material). It was only perceived on molecular analyzes. The juveniles resemble those of *Rubyspira* sp. nov. 1 but its shell is thinner.

Only 53 specimens of *Rubyspira* sp. nov. 2 were retrieved from landers, the least of all main species in abundance. Apart from rare species, it was the only gastropod collected just at 1500 m landers, and only at one lander (ES-1500). They were found in equal amounts on whale bones and wood parcels (26 individuals each). Only one animal occurred on the inert material and it could be due to mixing on samples during the rescue. Most collected specimens were juveniles, and few presumably fully grown adult were seen (Supplementary Material).

The larval shell presents 2-2.5 whorls, both axial and spiral sculptures, large larval shell compared to embryonic shell. These characteristics are common for gastropods with planktotrophic development. Its apical whorls are also autotomized and sealed with a calcareous, as for other *Rubyspira*. (Supplementary Material, Figure 2.11). The larval shell is commonly lost on larger specimens.

*Rubyspira* sp. nov. 2 also presents strong teeth, with finger-like structures in all its dentition that appears to be used to directly remove fragments from bones (Supplementary Material, Figure 2.9). It is also taenioglossa, as known for the group.

No stable isotopes analyze were conducted with *Rubyspira* sp. nov. 2, because of the lack of adult specimens, it would be necessary many juveniles to reach the necessary mass. Only few specimens were dissected to observe the gut contents, and only small particles of whale bone were found among sediment.

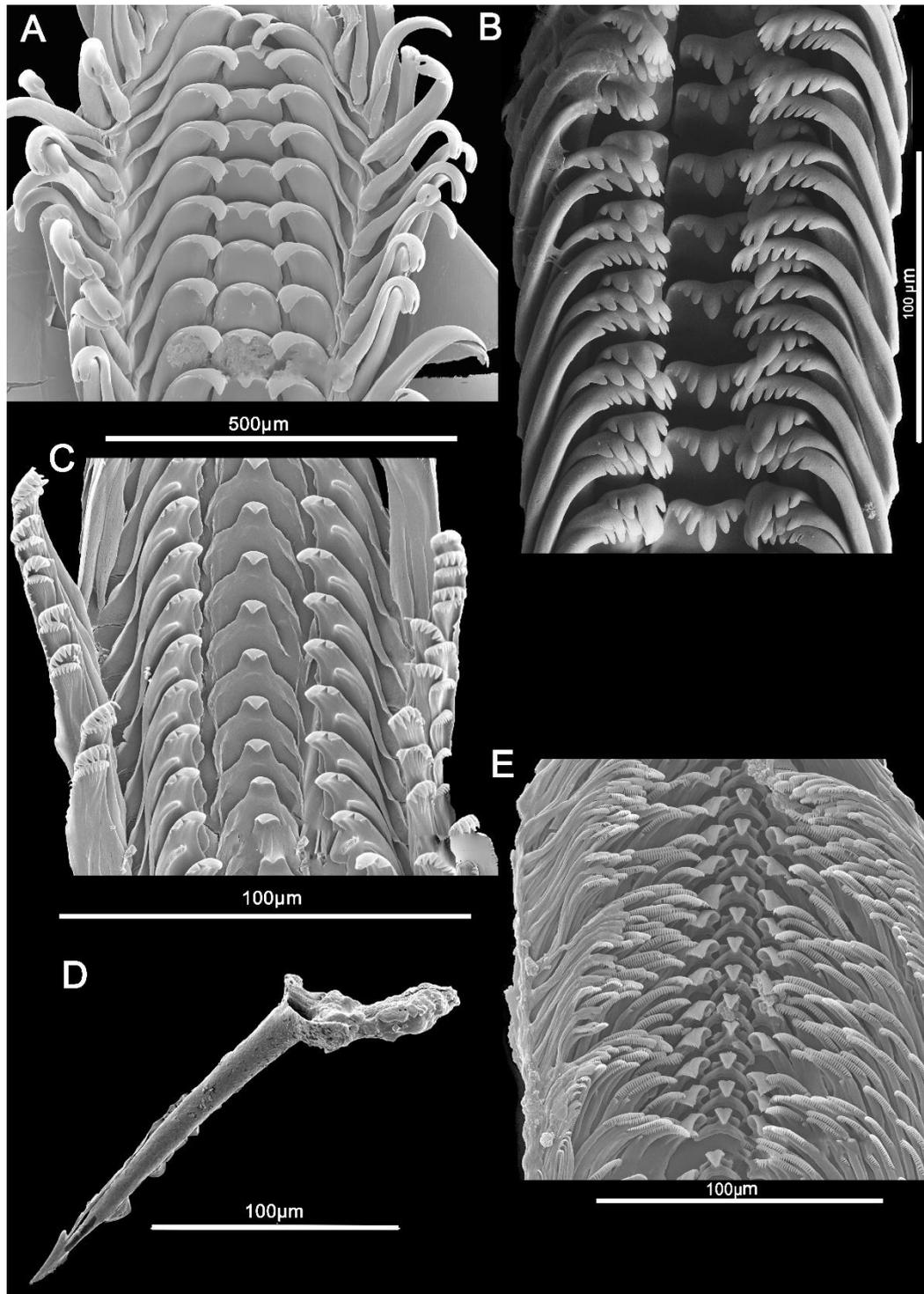


Figure 2.9: Scanning electron microscopy of radula teeth. A: *Rubyspira* sp. nov. 1, B: *Rubyspira* sp. nov. 2, C: *Cordesia* sp. nov., D: *Lusitanops cingulatus* radular tooth, E: *Hyalogyrina rissoela*.

Mollusca Linnaeus, 1758;  
Gastropoda Cuvier, 1797;  
Caenogastropoda Cox, 1959;  
Abyssochrysoidea Tomlin, 1927;  
Provannidae Warén and Ponder, 1991  
*Cordesia* Warén and Bouchet, 2009  
***Cordesia* sp. nov.**

The new species of *Cordesia* was described genetically and morphologically at Supplement material of this study. It is the first time that this genus was found dwelling on organic falls and on the Southwest Atlantic Ocean. Only 3 specimens of *Cordesia* were found until date on hydrocarbon seeps of West Africa.

It is the third most abundant gastropod, with a total of 164 specimens and were dominant both on wood parcels and on inert substrate representing 39.5% and 37.8% of gastropod fauna found respectively. But only 17.9% of total of *Cordesia* sp. nov. individuals used whale bones as substrate.

*Cordesia* sp. nov. was found only in 3300 m depth landers. They were settled mostly at RJ-3300 lander (62.5%). Animals of all sizes and stages of development were collected at 3300 m depth sites.

Planktotrophic traits could be observed on its larval shell, such as a large size compared to embryonic shell, axial and spiral well-defined sculptures and 2-2.5 whorls. Such as other provannid snails the apical whorls are autotomized and sealed with a calcareous plug, it appears to be slightly open when observed. It is the gastropod of the present study that presented most conserved protoconch I and protoconch II on adult animals (Supplementary Material, Figure 2.11).

Their radula resembles that of *Cordesia provannoides* that was described as a grazer on bacterial, such as other provannid snails. The marginal teeth are comb-like on the apex forming denticles, that are longer and less abundant on inner marginal tooth. Lateral teeth have a finger like structure on its side and the central have an expanded base (Supplementary Material, Figure 2.9).

The expected enrichment values of  $^{13}\text{C}$  and  $^{15}\text{N}$  generally relative to diet can be observed on stable isotopes results for *Cordesia* sp. nov. The former

corresponds to expected enrichment from a bacterial grazer, but not necessarily for the latter isotope. However, the digestive tracts of two specimens of *Cordesia* sp. nov. living on wood parcels had juvenile xylophagid bivalves within the sediment fund on their gut. Shell size and morphology of these bivalves suggest they were young settlers (Figure 2.10).

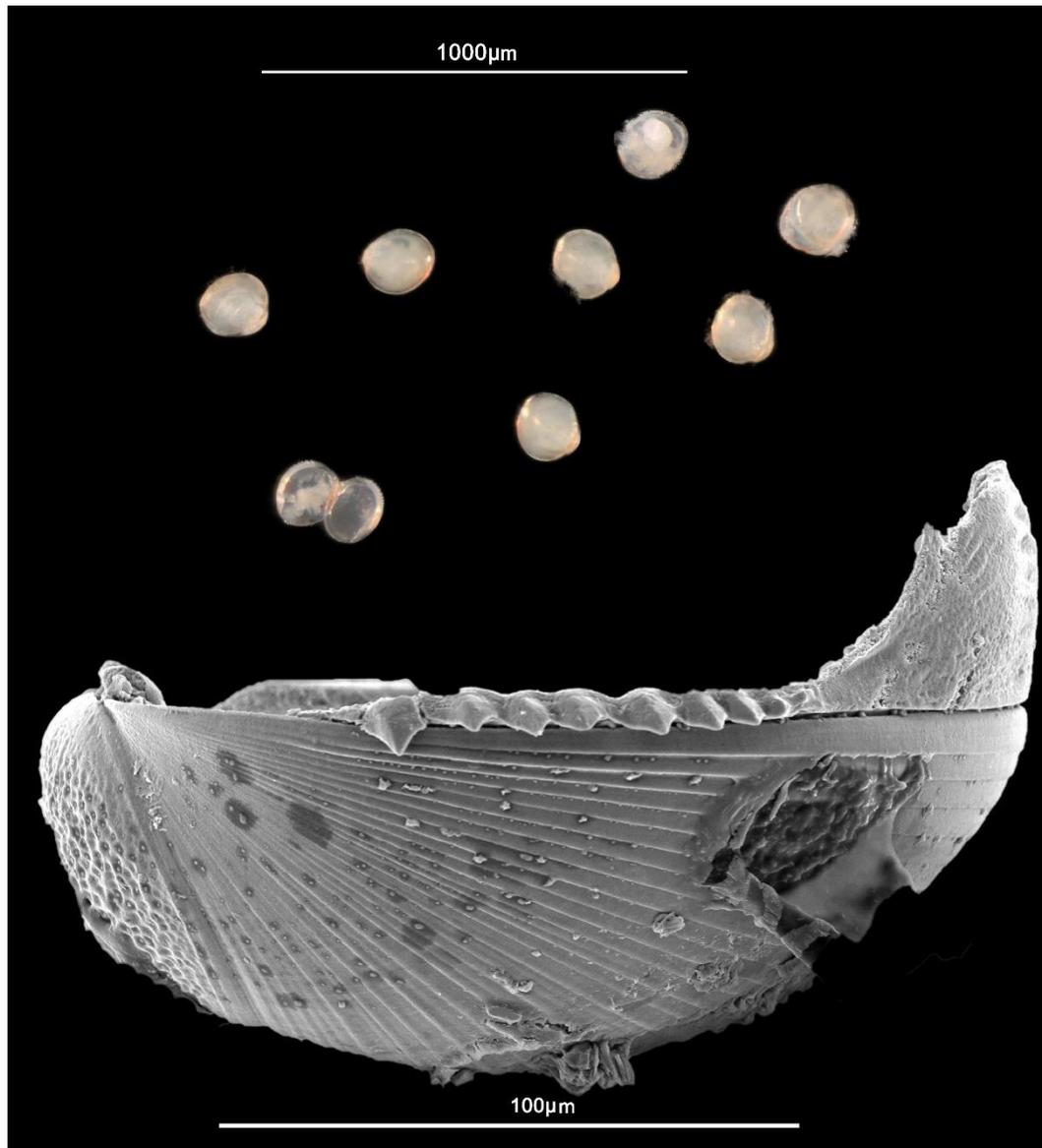


Figure 2.10: Scanning electron microscopy of a juvenile *Xylophagidae* bivalve that was in the digestive tract of an individual of *Cordesia* sp. Nov. Photograph under stereomicroscope of the same bivalves.

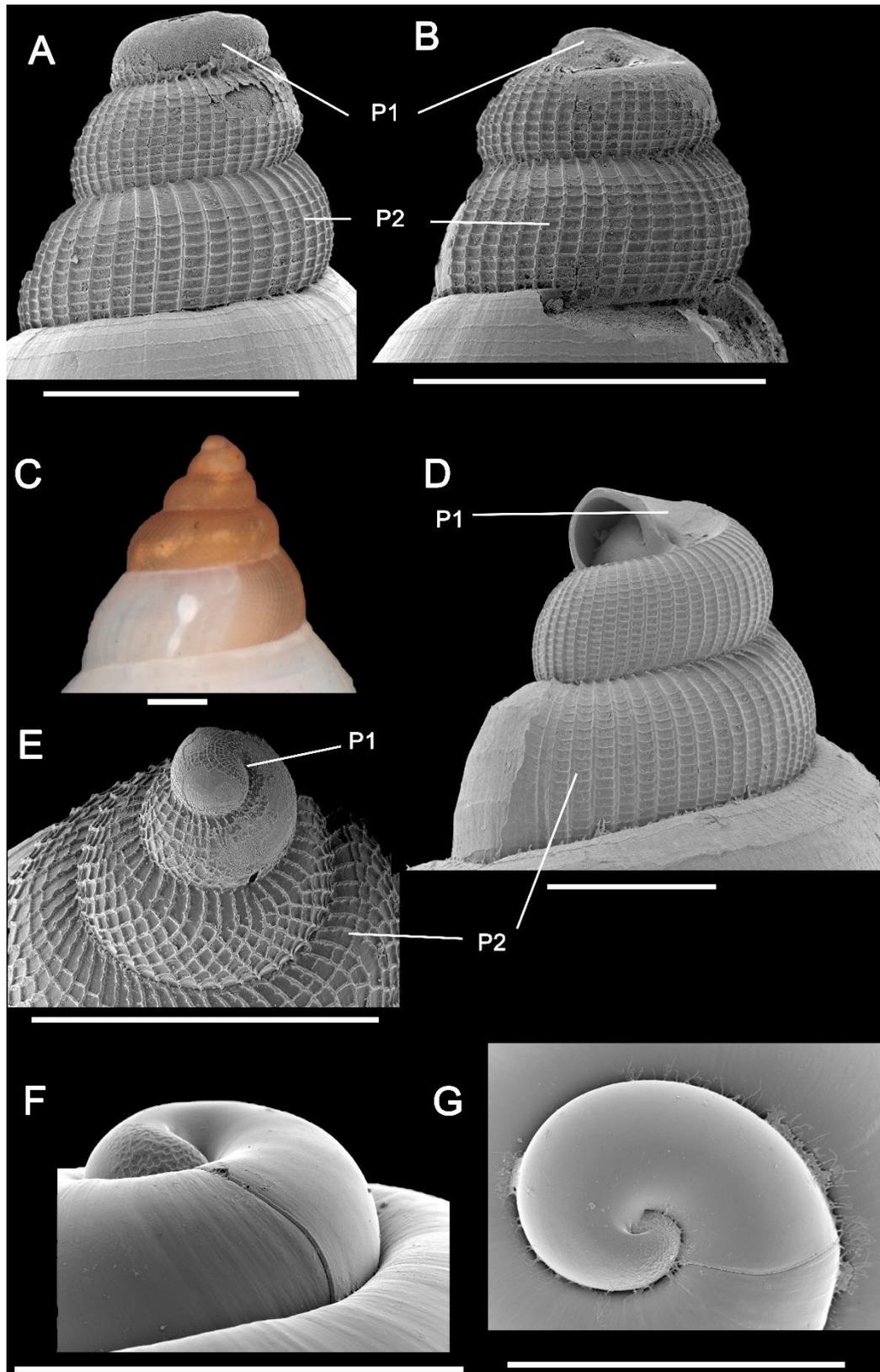


Figure 2.11: Scanning electron microscopy of larval shell of A: *Rubyspira* sp. nov. 1, B: *Rubyspira* sp. nov. 2, D: *Cordesia* sp. nov., E: *Lusitanops cingulatus*, F, G: *Hyalogyrina rissoela*. Stereomicroscope photo of *Lusitanops cingulatus* larval shell (C). P1 – Protoconch I, P2 – Protoconch II. *Cordesia* sp. nov., *Rubyspira* sp. nov. 1 and *Rubyspira* sp. nov. 2 protoconch I was lost and replaced by a calcareous plug. It is not possible to observe difference between protoconch I and II for *Hyalogyrina rissoela* specimens. All scales are 500µm.

Mollusca Linnaeus, 1758;  
Gastropoda Cuvier, 1797;  
Heterobranchia Burmeister, 1837;  
Valvatoidea Gray, 1840;  
Hyalogyrinidae Warén and Bouchet, 1993;  
*Hyalogyrina* Marshall, 1988;  
*Hyalogyrina rissoella* Warén and Bouchet, 2009.

This lower heterobranchia was described for the Southeast Atlantic Ocean on hydrocarbon seeps, and it is the first time it was found on food falls and on Southwest Atlantic basin. However, the Hyalogyrinidae are a recurrent group of chemosynthetic environments. There is no genetic data of this species, so molecular analyzes were conducted for the first time for *Hyalogyrina Rissoela*.

The COI and 16S for *Hyalogirina rissoela* phylogeny were not very elucidative at species level because of lack of molecular data of its family. The concatenated phylogenetic tree shows that this species is on a clade with high support but in a different branch than its own superfamily, Valvatoidea (represented by families *Valvatidae* and *Cornirostridea*). Nevertheless, the Superfamily Omalogyroidea and Architectonicoidea, that are on the same branch as *Hyalogirina rissoela*, seems to be farther apart (Supplementary Material). With COI alone the results are similar, a high supported clade with the same species, but this time *Hyalogyrina* is basal and the other Valvatoidea are closer. The 16S phylogenetic tree alone also showed a high supported clade but with the Valvatoidea species only.

The higher distance among *Hyalogyrina rissoela* specimens was smaller than 2.1%. However, for other species of Valvatoidea, they were between 72.4% and 81.8% similar (*Cornirostra pellucida* and *Valvata sibirica* respectively). The species of the Superfamily Omalogyroidea and Archtectonicoidea were the most distant group from *Hyalogirinidae*, with more than 40% identity difference (Supplementary Material).

*Hyalogyrina rissoella* was the most abundant species of the present study, summing 3775 individuals and was dominant on bones and at both depths. Only 17 specimens (0.5%) were on wood parcels, which may have been caused by mixing samples during the ascent of the lander. It represented 94.3%

of the gastropod fauna in SP-3300. Animals of all development stage were collected in every site.

This gastropod has less than one whorl on the larval shell. But the larval development of the genus is hard to infer, since no egg masses are known and even though the small size of protoconch, the absence of a well demarcated protoconch I unable comparisons to define planktotrophy or lecithotrophy (Figure 2.11).

The radula of *Hyalogyrina rissoela* is rhipidoglossate bearing series of comb-like teeth (more than thirty per line) resembling those of known grazer gastropod species. This species is known as a bacterial mats and detrital grazer and the form of its radula corroborates with it (Figure 2.9).

No gut content observations were held for this species, but literature indicates that most are filled with sediment. Results of stable isotopes presents the expected enhance on  $^{13}\text{C}$  and  $^{15}\text{N}$  for a grazer on bacterial mats.

Mollusca Linnaeus, 1758;  
Gastropoda Cuvier, 1797;  
Caenogastropoda Cox, 1959;  
Neogastropoda Wenz, 1938;  
Conoidea Fleming, 1822;  
Raphitomidae Bellardi, 1875;  
*Lusitanops* Nordsieck, 1968;  
*Lusitanops cingulatus* Bouchet and Warén, 1980.

This Neogastropoda was the most common gastropod of its group found on BioSuOr Project. It is described from sediment samples of Northeast Atlantic Ocean, for the Mediterranean Sea and for a natural whale fall of Southwest Atlantic Ocean. Raphitomidae are a well studied group of predators, however, there is no genetic data available for this species. Despite attempts to extract DNA from the material, it was not possible due to its level of degradation

*Lusitanops cingulatus* was the fourth species in terms of abundance. It occurred on all substrates, whale bone (57.9%), control (21.7%) and wood (20.4%). This indicates that they are probably opportunists on organic falls,

where they can rely on prey abundance. This mollusk was more common at 3300m depth, where 93.2% of all individuals were found, and gastropods of this species of all sizes were collected at different landers.

They present planktotrophic traits on larval shell, such as axial and spiral sculptures, many whorls on protoconch (2.5 - 3). The Raphitomidae also has a brownish larval shell that is a planktotrophic trait (Figure 2.11).

*Lusitanops cingulatus* radula is toxoglossal, highly modified with venom glands and radicular teeth (Figure 2.9) and it is a common feature of Neogastropoda. It is a clear indication of its predatory habits. Stable isotopes analyzes indicates that the enrichment of  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to diet are observed for *Lusitanops cingulatus*. Specially the nitrogen isotope, that is the highest mean among studied species.

## 2.4. DISCUSSION

The gastropod diversity found in the present study is the highest reported for both natural (Smith et al., 1989; Fujioka et al. 1993; Smith and Baco, 2003; Lundsten et al., 2010a; Amon et al., 2013; Smith et al., 2014; Sumida et al., 2016; Amon et al., 2016) and implanted organic falls (Smith and Baco, 2003; Fujiwara et al., 2007; Braby et al., 2007; Lundsten et al., 2010b). Gastropods were the most abundant epifaunal group, probably also in terms of biomass.

The bathymetric range of this study, associated with the diversity of substrates and the difference in latitude probably accounts for the species richness found (Braby et al., 2007). The time landers remained at the seabed can be the reason why SP-1500 had least species richness, since it was recovered almost 8 months earlier (Smith and Baco, 2003; Goffredi, 2004; Braby et al. 2007, Smith et al., 2014, Smith et al., 2015). This shorter time on seabed along with the circumstance during its rescue (i.e., sea roughness and ship restrictions) can also be the reason for low abundance of animals.

There are no available environmental data about disturbances that could influence richness and abundance, such as temperature changes, current speeds and dissolved oxygen variations on this study (Levin and Gage, 1998).

Together, deeper landers were more diverse and had more specimens. It is probably due to the rate of degradation of organic matter at deeper sites, which are lower due to mean temperature and pressure (Smith and Baco, 2003). Two of the main species were found only at one depth, *Rubyspira* sp. nov. 2 at 1500m and *Cordesia* sp. nov. at 3300 m. The genus of the former was found in higher depths (Johnson et al., 2010; Sumida et al., 2016; this study), but the latter genus was sampled just once from a similar depth (3150m) (Warén and Bouchet, 2009). All other gastropods with more than 40 specimens were found at both depths in this study.

Large number of individuals were associated with two main species living in whale bones, because both feed directly of this substrate or is indirectly benefited by the energetic input. The bone specialist *Rubyspira* sp. nov. 1. and *Hyalogyrina rissoela*, a bacterial carpet scraper (Warén and Bouchet, 2009)

dwelled on these substrates. The latter probably dwelled on whale bones because it was the substrate with most concentration of bacterial mats. Both organic substrates presented more species richness probably due to the carbon input provided by the organic enrichment (Smith and Baco, 2003; Smith et al., 2015).

Landers with higher abundance of gastropods were those in which *Hyalogyrina rissoela* was more common. ES-1500 and SP-3300, were both on sea floor for the same amount of time. There is no evidence for the preference of Heterobranchia for these sites. The only pattern observed was that these landers were where *Rubyspira* sp. nov. 1 smaller specimens were found, in contrast with RJ-3300 and ES-3300 with larger ones. Both species were dominant on this study.

There were no significant differences in species richness based on latitude, the only of the main species that appeared just in one latitude was *Rubyspira* sp. nov. 2 at ES-1500.

The bathymetric range for the genus *Rubyspira* increased its minimum from 1820 m (Johnson et al., 2010) to 1439 m, since *Rubyspira* sp. nov. 1 could be found on every station of this study. The maximum and minimum range for *Hyalogyrina rissoela* is now 1439-3358 m, in comparison to 3150 m depth reported by Warén and Bouchet (2009). Only the maximum depth for *Lusitanops cingulatus* changed, from the first reports on the Northeast Atlantic (Bouchet and Warén, 1980) and Mediterranean Sea (Bouchet and Taviani, 1989; Mutaf et al. 2008). Their presence at SP-3300 increases its maximum depth range to 3358 m, which was previously set at 2795 m (Bouchet and Warén, 1980; Bouchet and Taviani, 1989; Mutaf et al., 2008). *Cordesia* sp. nov. was found at 3358 m depth, increasing from the former type locality 3150 m (Warén and Bouchet, 2009).

Provannidae snails are often found in chemosynthetic environments, such as organic falls (Smith and Baco, 2003; Johnson et al. 2010; Lundsten et al., 2010a; Sumida et al., 2016, this study), hydrocarbon seeps (Warén and Bouchet, 2009), hydrothermal vents (Bouchet and Warén, 1991; Warén and Ponder, 1991; Warén and Bouchet, 1993; Suzuki et al., 2006b; Sasaki et al., 2010; Johnson et al., 2015; Sasaki et al., 2016), cold seeps (Kaim et al., 2008a; Sasaki et al., 2010; Sasaki et al., 2016) and serpentinite-hosted environment (Chen et al., 2016a,

2016b). The genus *Cordesia* was found only at hydrocarbon seeps in west Africa, and this is the first time it is reported on food falls. *Cordesia* is the second genus of Provannid that lives in more than one substrate, formerly only *Provanna* was known to do it (Sasaki et al., 2010). It was the first time that a gastropod from the family *Abyssochrysidae* was sampled at an organic fall, the specimens of *Abyssochrysos melanioides* inhabited all substrates in the present study (93.0% at whale bones).

It is also the first time that *Hyalogyrina rissoela* was reported for organic falls, but the genus is a recurrent organism on chemosynthetic habitats (Warén and Bouchet, 1993; 2001; 2009; Sasaki et al., 2010). Normally found in unconsolidated substrates, *Lusitanops cingulatus*, was found on whale bones before (Sumida et al., 2016), but it was not identified to species level and it was referred to as *Raphitomidae* sp. This is the first time that *Hyalogyrina rissoela* and the genus *Cordesia* were found in the Southwest Atlantic.

Both new species of *Rubyspira*, *Cordesia* sp. nov., *Lusitanops cingulatus* and *Hylogyrina rissoela* probably recruited more than once, since animals of all sizes and stages of development were found, suggesting larvae is available all year around at these areas.

*Lusitanops cingulatus*, *Rubyspira* sp. nov. 1, *Rubyspira* sp. nov. 2 and *Cordesia* sp. nov. presented planktotrophic characteristics on their larval shell morphology, and probably have this kind of development (Jablonski and Lutz, 1980; Jablonski and Lutz, 1983). On the other hand, *Hyalogyrina rissoella*, as Warén and Bouchet, (2009) stated, presents an uncertain type of larval development, following its genus, since no eggs of these animals were observed and the observation of protoconch I was difficult because there is no demarcated limit. Characteristics normally used to classify a gastropod in a planktotrophic or lecitotrophic species, such as shell sculptures, number of loops and size of protoconchs I and II were insufficient in this case (Jablonski and Lutz, 1983).

*Rubyspira* species was originally described as bone specialists (Johnson et al., 2010) and this is reinforced by the radula and gut content of both new species found in the present study. All other gastropods found dwelling on organic falls during this study seemed to be opportunistic mollusks, that take

advantage on the large input of food and associated community. *Lusitanops cingulatus*, for instance, are predators that use the organic falls probably owing to the availability of prey (Bouchet and Warén, 1980). *Cordesia* sp. nov. feed by scraping bacterial mats, what can justify they were more present at inert material (Warén and Bouchet, 2009) The juvenile bivalves found on its gut content suggests that they can exploit more than one type of food source as its suggested for deep sea snails (Sasaki et al., 2010). This supports the idea that deep-sea gastropods can use different food sources.

Isotopic data was very variable suggesting use of distinctive food sources (Sasaki et al., 2010). This is probably related to the different substrates and where animals were living. *Hyalogyrina rissoella*, a bacterial mat exploiter (Warén and Bouchet, 2009) exhibited the highest variation in isotopic values in consonance with the high isotopic variability found for its food source. The same applies for *Cordesia* sp. nov. that may have the same feeding behavior (Warén and Bouchet, 2009), but fed also of juvenile bivalves. *Lusitanops cingulatus* is a predator, and it is unlikely that it has only one food source (Bouchet and Warén, 1980).

*Rubyspira* presented the expected enrichment relative to diet for both isotopes (Johnson et al., 2010; Alfaro-Lucas et al., unpublished), but it is not clear whether the animal obtained carbon and nitrogen from the bones or from bacteria growing on it. However, the direct observation of guts filled with bones corroborates its diet on bones which can be enriched by chemosymbiotic bacteria (Johnson et al., 2010; this study).

This diversity on life habits is supported with conducted stable isotopes analyzes, gut content and radula observation (Sasaki et al., 2010). Due to many sources of nutrition, organic falls can support specialist and generalist animals, from multiple trophic levels what can enhance its species richness.

Faunal overlap among whale bones, wood parcels and other chemosynthetic environments were reinforced by the presence of *Hyalogyrina rissoella* and the genus *Cordesia*, both found previously on the Northeast Pacific Ocean and at hydrocarbon seeps off the Southeast Atlantic, concurring the

ecological "stepping stones" hypothesis (Smith et al., 1989; Distel et al., 2000; Smith and Baco, 2003; Samadi et al., 2007; Smith, 2015; Smith et al. 2017). This organic fall could play an ecological role helping organisms to disperse or could imply in evolutionary novelties in long periods of time. *Rubyspira* is a genus that have already been found at Southwest Atlantic Ocean (Sumida et al., 2016), raising to three the numbers of species for this Oceanic basin, and reinforcing the inter-basin distribution of bone specialists.

## CHAPTER 3

### 3. Molecular phylogenetics of Abysochrysoidea (Caenogastropoda: Mollusca)

#### 3.1. INTRODUCTION

Gastropods of the superfamily Abysochrysoidea are known for inhabiting the deep-sea and chemosynthetic habitats, such as hydrothermal vents, hydrocarbon seeps, organic falls such as wood and whale bones. Some of them may have used bone and wood as ecological and evolutionary “stepping stones” (Smith et al, 1989; Distel et al., 2000; Smith and Baco, 2003; Fujiwara et al., 2007; Samadi et al., 2007; Smith, 2015; Sumida et al., 2016; Smith et al. 2017). Some of these gastropods may dwell on more than one kind of chemosynthetic environment and were found in food falls, such as the genera *Provanna* (Smith and Baco, 2003) and *Cordesia* (this study).

Two families compose Abysochrysoidea, Abysochrysidae (Tomlin, 1927) and Provannidae (Warén & Ponder, 1991), the latter exclusive from chemosynthetic environments (Sasaki et al., 2010). They are often dominant in numbers and in biomass where they dwell (Bouchet and Warén, 1991; Smith et al., 2014; Johnson et al., 2010).

Abysochrysidae is monotypic, the genus *Abysochrysos* (Tomlin, 1927), is found mainly in deep-sea sediment and have never been found in chemosynthetic environments. They present carnivorous and parasitic behavior (Houbrick, 1979; Bouchet, 1991; Warén and Bouchet, 2009).

Provannids are placed into five genera, *Provanna* (Dall, 1918), *Alviniconcha* (Okutani and Ohta, 1988), *Ifremeria* (Bouchet and Warén, 1991), *Desbruyeresia* (Warén and Bouchet, 1993), and *Cordesia* (Warén and Bouchet, 2009). Besides, the genus *Rubyspira* (Johnson et al. 2010) is unassigned at family level, even though it is within the Abysochrysoidea.

*Alviniconcha* and *Ifremeria* are endemic to hydrothermal vents and present chemosymbiotic bacteria in their gills (Suzuki et al., 2006b). *Alviniconcha* is abundant in the Western Pacific and Indian Oceans, and all its species were first described as *Alviniconcha hessleri*, but later it was discovered that there were five distinct cryptic species (totalizing 6 species) (Johnson et al., 2015). *Ifremeria nautiliei* is monotypic, and occurs at Manus, North Fiji and Lau basins. Organisms of these both genus are large gastropods, with more than 50 mm in shell height, that rely on endosymbiotic sulfur-oxidizing bacteria for their nutrition (Bouchet and Warén, 1991; Johnson et al., 2010).

The largest genus is *Provanna*, containing 22 described species and a cosmopolitan distribution. They were found in most deep-sea chemosynthetic habitats, such as hydrothermal vents, cold seeps and organic falls. They are medium sized snails (generally less than 15 mm in shell height), that are mostly deposit feeders and bacterial mat grazers (Warén and Bouchet, 1993; Sasaki et al., 2010). *Provanna cingulata* was the first abyssochrysoid snail found on serpentinite-hosted ecosystems (Chen et al., 2016a) along with a *Desbruyeresia* species (Chen et al., 2016b). *Desbruyeresia* encompasses six described species, five of them are known from hydrothermal vents from Western Pacific and Indian Oceans (Kaim et al., 2008a; Sasaki et al., 2010), together with a specie from serpentinite-hosted vents (Chen et al., 2016b).

*Cordesia provannoides* is also monotypic, and was described from a hydrocarbon seep off Congo, West Africa, using only one specimen. Since then, only three individuals of this group were collected (Warén and Bouchet, 2009). In the present study, a new species of *Cordesia* was discovered, in wood and whale falls. There is still an open question whether this genus should be classified as an Abyssochrysoidea or Provannidae, since it is the only gastropod of its family that presents a penis, which is considered plesiomorphic feature and it was lost in other provannid snails. This fact opens another hypothesis that Provannidae should be included in Abyssochrysoidea (Warén and Bouchet, 2009). However, no molecular analyses were made for *Cordesia provannoides* due to the lack of material.

The last genus of Abysochrysoidea is *Rubyspira*, with two formally described species (Johnson et al., 2010), and another species from the Southwest Atlantic yet to be described (see Sumida et al., 2016). Two more new species were found and described in this study, all five of them specialists and endemic to whale falls. *Rubyspira* seems to feed directly on whale bones or in bone enriched sediments, judging from gut content and stable isotope analysis (Johnson et al., 2010; this study). All these species are numerous on the places they are found, representing a significant biomass of the fauna in whale falls. They are large gastropods and can reach more than 40 mm in shell height (Johnson et al., 2010; Sumida et al., 2016; this study). Neither of the already described *Rubyspira* species resembles recent abyssochrysoid snails. *Rubyspira osteovora*, *Rubyspira* sp. nov. 1 and *Rubyspira* sp. nov. 2 resemble the fossils of *Atresius liratus* (Gabb, 1869; Kiel et al., 2008), that occurred at Cretaceous hydrocarbon seeps (Kiel et al., 2008). *Abysochrysoidea* is *Rubyspira* closest living gastropod, sharing some morphological traits and corroborated by close phylogenetic proximity (Johnson et al., 2010). Nevertheless, the genus *Rubyspira* is still unassigned at family level, what demands more investigation to properly establish it.

The oldest report for snails of the Provannidae family are from the Upper Jurassic to Lower Cretaceous hydrothermal vents. However, to use them as indicative of the appearance of this family is speculative due to its bad conservation state. The safest fossil record of these gastropods is *Provanna antiqua* (Squires, 1995) from the Eocene–Oligocene. For Abysochrysoidea, the first reliable record is the genus *Hokkaidoconcha* from the Late Cretaceous, indicating that this superfamily has been around for 131 to 145 million years, an old lineage (Kaim et al., 2008a). Molecular evidence indicates a quick radiation of this group during the mid-Cretaceous, and the start of its diversification during the Mesozoic (Johnson et al., 2010). The discovery of Late Cretaceous abyssochrysoid shells associated with a plesiosaurid carcass indicates that these large reptiles could support communities such as those found at modern whale falls (Smith and Baco, 2003; Kaim et al., 2008a, b).

The phylogenetic position of the family Provannidae within Caenogastropoda is still uncertain, but it was used as a sister group of the Littorinidae by Colgan et al. (2007) and are hypsogastropods (Ponder and Lindberg, 1997). However, only one species of Provannidae was included, and no Abysochrysoidea. Kaim et al. (2008a) associated some of the fossil specimens described as *Hokkaidoconcha* as being possibly of the superfamily Cerithioidea, but the relationship between these families are not supported by genetic studies (Colgan et al., 2007). Morphological analyses indicate that provannids were part of the superfamily Abysochrysoidea (Warén and Bouchet, 2009), what was later reinforced by molecular data by Johnson et al. (2010) and Chen et al. (2016a, 2016b). Provannidae is probably paraphyletic because the Abysochrysoidea is rooted at the same clade, making the genus *Rubyspira* to remain unassigned at family level (Johnson et al., 2010).

The clade including *Rubyspira*, *Desbruyeresia* and *Abysochrysoidea* is well supported on other phylogenetic studies and seems to have radiated 26-90 million years ago. The same could be said for the basal clade formed by the genus *Provanna* alone and a third branch with species of *Alviniconcha* and *Ifremeria nautilei* (Johnson et al., 2010; Chen et al., 2016a, 2016b).

With the available data for multi locus (COI, 16S, 18S and 28SD1) provided by Johnson et al. (2010, 2015) and the study made by Colgan et al. (2007) with Caenogastropoda and considering the new described species of *Rubyspira* and *Cordesia* in the present study, the objectives of this work are to properly classify the genus *Rubyspira* in a Family, to add molecular data to the genus *Cordesia*, and to analyze the possible phylogenetic position of the Superfamily Abysochrysoidea within the Caenogastropoda.

### 3.2. MATERIAL AND METHODS

Samples were collected during the BioSuOr Project, and initially preserved in molecular ethanol and frozen at -80°C on board. DNA was extracted from foot muscle, using the DNeasy Blood and Tissue Extraction Kit (QIAGEN) of at least three individuals of each species of *Rubyspira* and *Cordesia*.

Table 3.1. Primers used in the investigation

Locus	Primers	Forward	Reverse	Length (bp)	References
Cytochrome-c-oxidase subunit-I (COI)	HCO/LCO	TAAACTTCA GGGTGACCA AAAAATCA	GGTCAACA AATCATAA AGATATTG G	~ 650	(Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994)
16S mitochondrial RNA	16SAR/BR	CGCCTGTTTA TCAAAAACA T	CCGGTCTGA ACTCAGAT CACGT	~ 550	(Palumbi, 1996)
28S ribosomal RNA subunit-D1	28SD1F/R	ACCCSCTGA AYTTAAGCA T	AACTCTCTC MTTCARAG TTC	~ 350	(Colgan, Ponder, & Eggler, 2000)
18S ribosomal RNA	18S1F/4R	TACCTGGTTG ATCCTGCCAG TAG	GAATTACC GCCGCTGCT GG	~ 550	(Giribet, Carranza, Baguna, Riutort, & Ribera, 1996)

Specimens were used for sequencing the mitochondrial cytochrome oxidase c subunit I (COI mtDNA) gene, 16S ribosomal RNA (16SrRNA), the 18S rRNA and 28S rRNA-D1. Around 650 bp of COI were amplified by polymerase chain reaction (PCR) using the primers HCO and LCO (Folmer et al., 1994), around 550 bp of 16S rRNA with 16SAR and 16SBR primer (Palumbi, 1996), 550 bp of 18S rRNA with 18S1F and 18S4R (Giribet, et al., 1996) and with the primer 28SD1F and 28SD1R approximately 350 bp of 28S rRNA-D1 were amplified (Colgan, et al., 2000) (Table 3.1.). The PCR was carried out in a 12.5 µl reaction, including 2 µl of DNA template, 0.1 µl of both reverse and forward primers (20 µM), 6.25 µl Gotaq Mastermix dNTP mixture 2x, and 4.05µl of deionized sterilized water. The thermocycling protocol was

initialized at 95°C for 1 minute for desnaturation, followed by 35 cycles of 45 seconds at 94°C, annealing at 50°C for 1 minute, an extension step at 72°C for 1 minute, ending with 72°C for 7 minutes. The amplification was checked through a 1.2% agarose gel electrophoresis. The purification was conducted with the QIAGEN Purification Kit.

Bidirectional sequences were aligned and properly edited checking its accuracy using the software Geneious (v11.0.3). Phylogenetic analyses and alignments were carried using the same software, the resulting sequences and the abyssochrysoid COI, 16S, 18S and 28S sequences available on GenBank provided by Johnson et al. (2010). Some groups near to this superfamily as pointed by Colgan et al. (2007) and the GC Group (gastropods with inhalant siphons) of littorinimorpha were selected. Other Caenogastropoda included in the genetic analyses were *Maoricolpus roseus*, *Pomacea bridgesii* and *Campanile symbolicum*. These organisms do not belong to Littorinimorpha and their phylogeny is still considered doubtful. *Neptunea antiqua* was selected as representative of Neogastropoda, a well defined monophyletic clade within the Caenogastropoda (Strong, 2003). The lower heterobranch *Cornirostra pellucidais* was used as outgroup, since the Heterobranchia are considered a sister group of Caenogastropoda (Ponder and Lindberg, 1997) (Table 3.2).

The model for tree constructions was selected using JModelTest software with BIC criteria, and the Bayesian analyses were made using the MrBayes v3.2.6 for each locus individually and in a final concatenated tree. Phylogenetic trees were also made using RaxML, for maximum likelihood, with both gamma and invariable sites.

Table 3.2.: Classification and publication data of the studied species locus. Gene segments not included in the data are indicated by blank cells. \* Bouchet and Rocroi (2005), but this group is poorly studied and the name Cerithiopsidae is chosen to use the better-known name.

Higher taxon	Family	Species	COI	16S	18S	28SD1
Heterobranchia						
Valvatoidea	Comirostridae	<i>Cornirostra pellucida</i>	Dinapoli, 2009	Dinapoli, 2009	Dinapoli, 2009	Dinapoli, 2009
Neogastropoda						
Buccinoidea	Buccinidae	<i>Neptunea antiqua</i>	Johnson et al., 2010			
Caenogastropoda						
Abyssochrysoidea	unassigned	<i>Rubyspira osteovora</i>	Johnson et al., 2010			
Abyssochrysoidea	unassigned	<i>Rubyspira goffredi</i>	Johnson et al., 2010			
Abyssochrysoidea	unassigned	<i>Rubyspira sp. Nov.</i>	This study	This study	This study	This study
Abyssochrysoidea	unassigned	<i>Rubyspira sp. Nov.</i>	This study	This study	This study	This study
Abyssochrysoidea	Provannidae	<i>Ifremeria nautilei</i>	Johnson et al., 2010			
Abyssochrysoidea	Provannidae	<i>Cordesia sp. Nov.</i>	This study	This study	This study	This study
Abyssochrysoidea	Provannidae	<i>Alviniconcha adamantis</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Alviniconcha boucheti</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Alviniconcha hessleri</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Alviniconcha kojimai</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Alviniconcha marisindica</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Alviniconcha strummeri</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Debruyeresia melanioides</i>	Johnson et al., 2010			
Abyssochrysoidea	Provannidae	<i>Provanna buccinoides</i>	-	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010
Abyssochrysoidea	Provannidae	<i>Provanna ios</i>	Johnson et al., 2010			
Abyssochrysoidea	Provannidae	<i>Provanna laevis</i>	Johnson et al., 2010			
Abyssochrysoidea	Provannidae	<i>Provanna lomana</i>	Johnson et al., 2015			
Abyssochrysoidea	Provannidae	<i>Provanna macleani</i>	Johnson et al., 2010			

Higher taxon	Family	Species	COI	16S	18S	28SD1
Abyssochrysoidea	Provannidae	<i>Provanna pacifica</i>	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010
Abyssochrysoidea	Provannidae	<i>Provanna sculpta</i>	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010
Abyssochrysoidea	Provannidae	<i>Provanna variabilis</i>	-	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010
Abyssochrysoidea	Abyssochrysidae	<i>Abyssochrysos melanioides</i>	Takano, 2014	Takano, 2014	Takano, 2014	Takano, 2014
Abyssochrysoidea	Abyssochrysidae	<i>Abyssochrysos melvilli</i>	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010	Johnson et al., 2010
Littorinoidea	Littorinidae	<i>Littorina Littorea</i>	Blakeslee et al., 2008	Giribet et al., 2007	Giribet et al., 2007	Giribet et al., 2007
Littorinoidea	Littorinidae	<i>Austrolittorina unifasciata</i>	Colgan et al., 2007	Criscione, 2013	Colgan et al., 2007	Colgan et al., 2007
Pterotracheoidea	Pterotracheidae	<i>Pterotrachea coronata</i>	Colgan et al., 2007	-	-	Colgan et al., 2007
Cingulopsoidea	Eatoniellidae	<i>Estoniella atropurpurea</i>	Wilke et al., 2012	Wilke et al., 2012	Wilke et al., 2012	Criscione, 2013
Triphoroidea	Cerithiopsidae	<i>Ataxocerithium sp.</i>	Colgan et al., 2007	-	Colgan et al., 2007	Colgan et al., 2007
Vermetoidea	Vermetidae	<i>Thylacodes adamsii</i>	Zou et al., 2011	Zou et al., 2011	Zou et al., 2011	-
Naticoidea	Naticidae	<i>Conuber melanostoma</i>	Colgan et al., 2007	-	Colgan et al., 2007	Colgan et al., 2007
Ampullarioidea	Ampullariidae	<i>Pomacea bridgesii</i>	Colgan et al., 2007	Criscione, 2013	Colgan et al., 2007	Colgan et al., 2007
Cerithioidea	Turritelidae	<i>Maoricolpus roseus</i>	Colgan et al., 2007	Donald, 2014	Colgan et al., 2007	Colgan et al., 2007
Campaniloidea	Campanilidae	<i>Campanile symbolicum</i>	Colgan et al., 2007	-	Colgan et al., 2007	Colgan et al., 2007

### 3.3. RESULTS

The alignments used in the molecular analyses had 1800 base pairs, and most sequences presented the four loci (COI, 16S, 18S and 28SD1). Two did not have COI in the database, *Provanna buccionoides* and *Provanna variabilis*. *Thylacodes adamsii* 28S sequence was also absent. No 16S sequences were available for *Campanile symbolicum*, *Conuber melanostoma*, *Ataxocerithium* sp. and *Pterotracheia coronata*. No 18S sequences were available *Pterotracheia coronata* for on the database.

#### Phylogenetic analyses of the single-gene datasets

Individual gene data rarely recovered well-supported higher taxa. Hypsogastropoda was seen in Bayesian analyses of COI and 16S (excepting Vermetidae). For maximum likelihood, 28S and 16S analyses weakly supported a Hypsogastropoda clade. The Neogastropoda, *Neptunea antiqua*, never had a clade of its own, and was rooted in Hypsogastropoda.

The superfamily Abysochrysoidea was not supported in any individual gene in the Bayesian analyses. They appeared only in 18S and 28S using maximum likelihood. However, *Ataxocerithium* sp. (Cerithiopsidae) was included and were very poorly supported in both analyses. The families Provannidae and Abysochrisidae were never observed. All genus of the Abysochrysoidea formed clades of their own using Bayesian and maximum likelihood for COI and 16S.

#### Phylogenetic analyses of the combined dataset

The topologies for Maximum likelihood and Bayesian inference yielded similar results (Figure 3.1, Figure 3.2). The Abysochrysoidea were present in both trees, with minor variation. The Superfamily Cerithioidea was distant from the Abysochrysoidea.

For maximum likelihood there was a low supported clade for the superfamily. A clade composed of the genera *Alviniconcha* and *Ifremeria* is basal within the Abysochrysoidea. A phylogenetically more diverse clade

including *Abyssochrysos*, *Rubyspira* and *Cordesia*. *Provanna* and *Desbruyeresia* cluster together in one clade. In this analyze, *Ataxocerithium* sp. is a sister clade to Abyssochrysoidea. The Neogastropoda *Neptunea antiqua* appears as a sister group of all Caenogastropoda and there was no evidences of the Hypsogastropoda on this analysis.

In the Bayesian inference topology, the Abyssochrysoidea clade is well supported, and so are the genera of the group. The results are similar to maximum likelihood analyze, excepted *Debruyeresia* that did not present enough support to cluster with *Provanna*. There is no evidence for a more basal clade. *Ataxocerithium* sp. is within Abyssochrysoidea with high support on this analysis. The Architaenioglossa (*Pomacea bridgesii*), the Cerithioidea (*Maoricolpus roseus*) and the Campanilidae (*Campanile symbolicum*) are the basal Caenogastropoda. Besides the presence of the Neogastropoda *Neptunea antiqua*, a well supported clade of Hypsogastropoda can be seen.

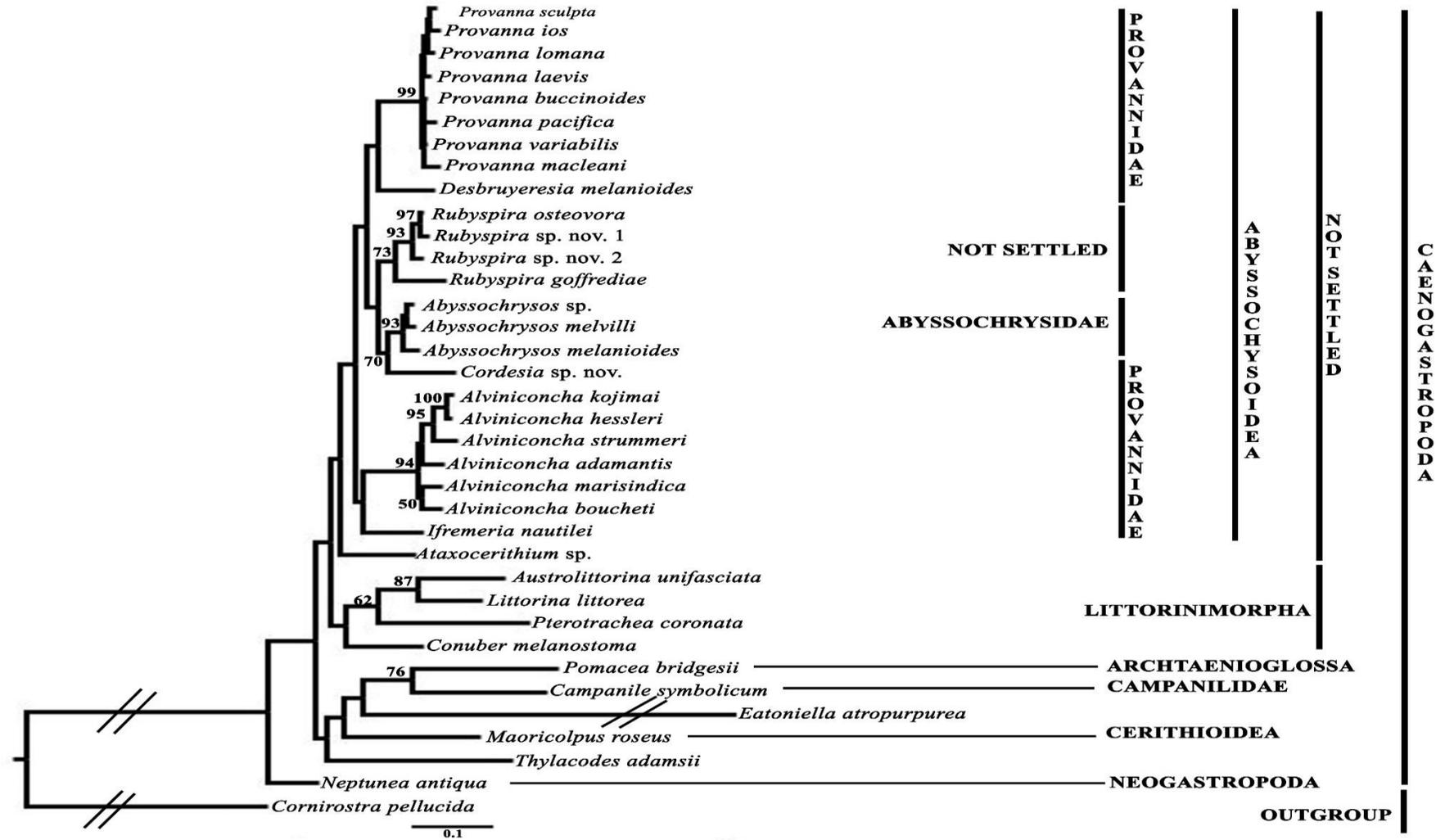


Figure 3.1: Concatenated phylogenetic tree (COI, 16S, 18S and 28SD1) of Caenogastropoda including Abyssochrysoidea superfamily. Maximum likelihood tree (GTR +G +I). Bayesian inference. Support level probability are given above branches. Named higher taxa are named by bars to the right of topology. *Cornirostra pellucida* and *Eatoniella* branches were cut for better resolution.

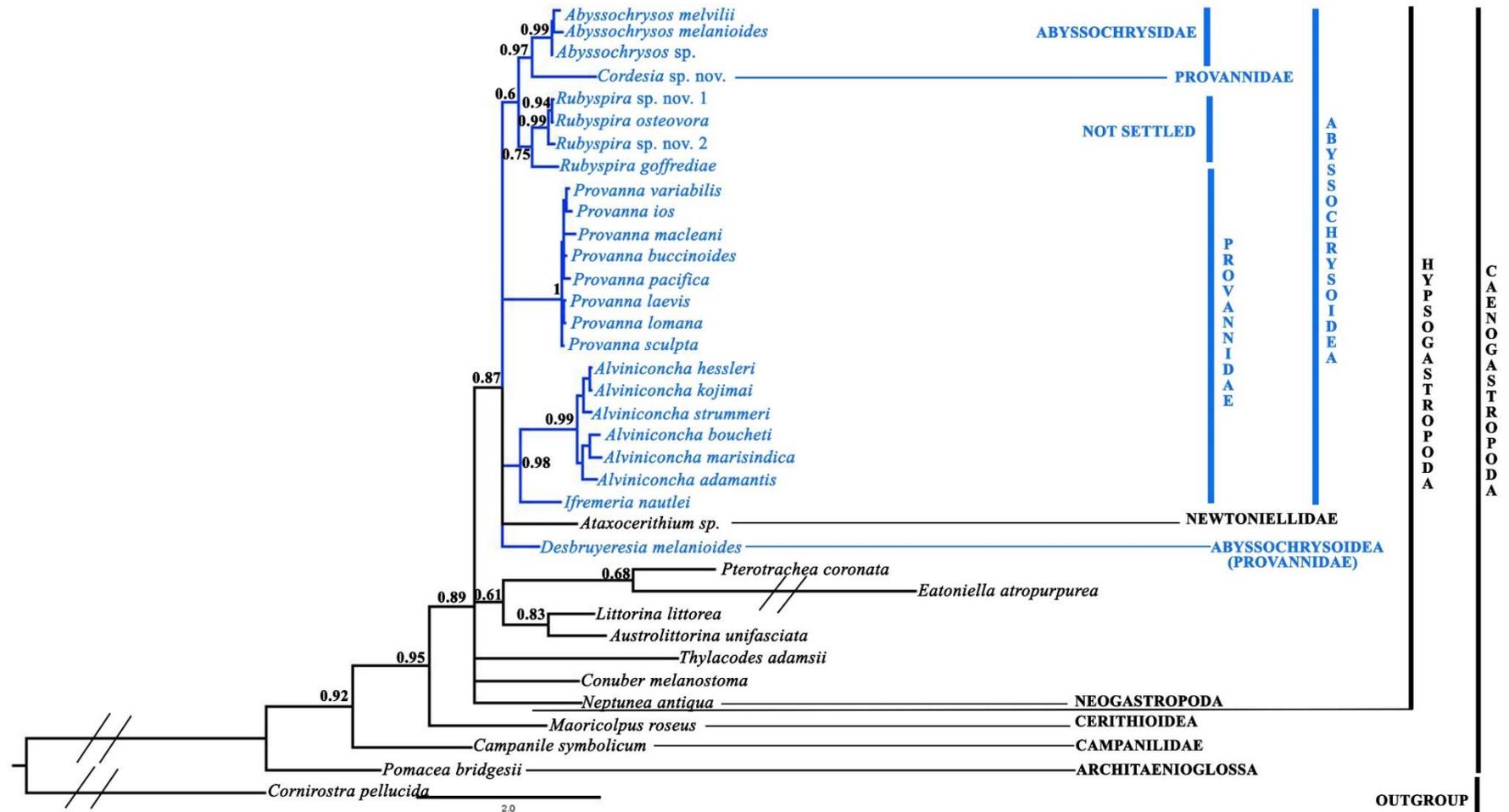


Figure 3.2: Concatenated phylogenetic tree (COI, 16S, 18S and 28SD1) of Caenogastropoda including Aabysochrysoidea superfamily (blue). Bayesian inference (G +I). Support level probability are given above branches. Named higher taxa are named by bars to the right of topology. *Cornirostra pellucida* and *Eatoniella* branches were cut for better resolution.

### 3.4. DISCUSSION

The data available on Caenogastropoda allowed the investigation of the position of the superfamily Abysochrysoidea in this group. In all analyses where there was the presence of Hypsogastropoda, the Abysochrysoidea were rooted in it. Colgan et al. (2007) pointed the family Provannidae as a sister group of Littorinidae based on 16S results. However, in the present study all analyses suggested *Ataxocerithium sp.* (Cerithiopsidae) as the closer group, most of times as a sister clade. This group may be closer to *Provannidae* and *Abysochrysoidea* than Littorinidae. This is uncertain since this group is not well studied and should be treated as Newtoniellidae (Bouchet and Rocroi, 2005) for better a association with the Abysochrysoidea.

The Abysochrysoidea clade supported that Provannidae is probably a paraphyletic group because the genera *Abysochrysoidea*, *Rubyspira* and *Cordesia* were always clustered together (Warén and Bouchet, 2009; Johnson et al., 2010; Chen et al. 2016a; 2016b). Alternatively, *Cordesia* may be classified as Abysochrysoidea instead of Provannidae since it is supported by the first molecular analyses of the genus and by its morphological similarities and genetic distances with the genus *Abysochrysoidea*, such as happened with *Rubyspira* (Warén and Bouchet, 2009; Johnson et al., 2010).

This study supports that a morphological family level revision should be undertaken. The hypothesis that Provannidae should be included in the Abysochrysoidea (Warén and Bouchet, 2009) should be considered. Another hypothesis that more than two Families should be included within the Abysochrysoidea, since the genetic distances among the genera are often higher than among other families.

The abysochrysoidea genera appear to be robust. *Abysochrysoidea* (Tomlin, 1927), *Provanna* (Dall, 1918), *Alviniconcha* (Okutani and Ohta, 1988), *Ifremeria* (Bouchet and Warén, 1991), *Desbruyeresia* (Warén and Bouchet, 1993), *Cordesia* (Warén and Bouchet, 2009) and *Rubyspira* (Johnson et al. 2010) are well supported morphologically and genetically. This study agrees with clades found in other molecular analyses of the superfamily, which cluster the

genus *Ifremeria* with *Alviniconcha*, *Rubyspira* with *Abyssochrysos* (and now *Cordesia*) (Johnson et al., 2010; Chen et al. 2016a).

However, the position of *Provanna* and *Desbruyeresia* remains uncertain, since it was not possible to find similar results of other studies. *Provanna* is not a basal clade on this study and often fall on the same clade as *Desbruyeresia*. Most of times the clade with *Ifremeria* and *Alviniconcha* was basal.

*Cordesia* and *Rubyspira* seems to be more related with the genus *Abyssochrysos* than to other provannid. The main difference seems to be ecological, since no Abyssochrysoidea was found before on a chemosynthetic environment (see this study). Thereby *Cordesia* species should be reallocated and *Rubyspira* should be settled as Abyssochrysoidea or a large revision of the superfamily should be undertaken. If the idea that Abyssochrysoidea have more than two families is correct, *Cordesia* should stay on Abyssochrysoidea due to morphological and genetic similarities, and *Rubyspira* is uncertain or even in a new family.

The clade composed by *Rubyspira*, *Cordesia* and *Abyssochrysos* were never basal in Abyssochrysoidea. This branch is always terminal and it is the only one that has a bone specialist (*Rubyspira*) and gastropods that were not found earlier on chemosynthetic environments (*Abyssochrysos*) (Houbrick, 1979; Bouchet, 1991; Warén and Bouchet, 2009; Johnson et al., 2010). Besides *Rubyspira*, only *Cordesia* (this study) and *Provanna* (Smith and Baco, 2003; Lundsten et al., 2010a) were found on whale bones. This suggests that the evolutionary novelty of organisms using whale falls as “stepping stones” hypothesis may not have happened for this group of mollusks. The idea that deep-sea snails started to colonize food falls, then through speciation started to inhabit more reduced environments (i.e. hydrothermal vents) (Smith et al, 1989; Distel et al., 2000; Smith and Baco, 2003; Fujiwara et al., 2007; Samadi et al., 2007; Smith, 2015; Sumida et al., 2016; Smith et al. 2017) seems inverted in this case.

It seems that, when the Abyssochrysoidea speciated from hydrothermal vents the genus *Rubyspira* became a bone specialist, and Abyssochrysos started to inhabit non-chemosynthetic environments. The evolutionary “stepping stones” hypothesis seems to apply for *Rubyspira* and *Abyssochrysos*, but with hydrothermal vents serving as “stepping stones”.

## CHAPTER 4

### 4. FINAL REMARKS

This study aimed to (1) understand the gastropod fauna of whale and wood falls artificially implanted by the BioSuOr Project, its composition, distribution, species richness, life habits and compare it with other better known oceanic basins (i.e. Northeast Pacific Ocean), since gastropods have an important contribution to these environments owing to their abundance; (2) to better understand the phylogeny of Abysochrysoidea, a superfamily of gastropods that dwells on chemosynthetic environments, specially the bone eater *Rubyspira* which remains to be assigned at family level; and (3) to check if the “stepping stones” hypothesis apply for these animals.

Gastropods were very abundant and had a high species richness. A total of 14 species were collected that summed 5493 specimens, one of the most abundant fauna of the project. Three of the mollusks from the Abysochrysoidea were new species described in this study (Supplementary material). Two *Rubyspira*, a bone specialist, and a *Cordesia*, a bacteria mat grazer were studied. The former had already been found in the Southwest Atlantic Ocean on a natural whale fall (Sumida et al., 2016), and the latter one was known only from hydrocarbon seeps on Southeast Atlantic Ocean (Warén and Bouchet, 2009).

Two more species were also very abundant and considered relevant ecologically. The raphitomid snail *Lusitanops cingulatus*, a predator, described for the Northeast Atlantic, probably taking advantage of the availability of food sources found in different (Bouchet and Warén, 1980). *Hyalogyrina rissoela*, a bacterial grazer heterobranch, was the most abundant species, it was formerly only known from hydrocarbon seeps of West Africa (Warén and Bouchet, 2009). The genus *Hyalogyrina* is recurrent in chemosynthetic environments (Warén and Bouchet, 1993; 2001; 2009; Sasaki et al., 2010), and it was found for the first time on whale carcasses and wood parcels. The bathymetric range of five main gastropods was augmented.

Depth and substrate type seemed to be the main factor for high abundance and species richness. Furthermore, the time lander remained at the sea floor was relevant for both abundance and species richness (i.e. lander SP-1500). Latitude did not influence in any of these parameters.

The discovery of two new species of *Rubyspira* are the second record for the genus outside of Monterey Canyon, suggesting they can occur worldwide. The second time *Lusitanops cingulatus* was found on this basin (see Sumida et al., 2016), with the first time a gastropod of the family Abysochrysoidea was found on whale carcass and summed with the findings of *Hyalogyrina rissoela* and *Cordesia* for the first time dwelling on food falls and on the Southwest Atlantic contribute to the ecological role of whale and wood falls as “stepping stones” for organism dispersal, suggesting that dispersal plays a major role on diversification on organic falls (Smith et al., 1989; Distel et al., 2000; Smith and Baco., 2003; Samadi et al., 2007; Smith, 2015; Smith et al., 2017).

Gastropods found on the present study presented many life habits, such as a variety of feeding behavior, larval development and morphological characteristics. This plasticity may have contributed to their survival of extinct events that affected shallow-water taxa and enabling diversification.

Phylogenetics with deep-sea groups are difficult, most of times due to lack of available data of animals, which are caused by lack of sampling and low numbers of specimens to do many analyzes. *Hyalogyrina rissoela* and *Lusitanops cingulatus* were both already described species without DNA data. Studies were held for both species and no results were possible for the Raphitomidae because of its level of degradation. The results for the heterobranch snail will be available on GenBank as soon as possible. However, it was not enlightening on species level, since there is no data of Hyalogyrinidae, but it could be placed on a superfamily, the Valvatoidea, along Valvatidae and Cornirostridae, what is supported by morphology and genetic distances.

With the data available of the Abysochrysoidea and Caenogastropoda, mostly provided by Johnson et al. (2010) and Colgan et al. (2007), and the data of the 3 new species of this study (will be deposited on GenBank) was possible

to infer that the Abyssochrysoidea are Hypsogastropodas (Ponder and Lindberg, 1997) supported by morphology and most of phylogenetic analyzes of the present study. However, *Ataxocerithium* sp. seems to be from a closer group than the Littorinidae. *Ataxocerithium* is a genus of the poorly studied family Newtoniellidae and was considered a Cerothiopsidae for better understanding on previous studies (Colgan et al., 2007). All tree reconstructions and genetic distance strongly supported this close affinity between Abyssochrysoidea and Newtoniellidae, but morphological work should be done.

*Rubyspira* was always on the same clade as the genus *Abyssochrysos* and are also the most similar living gastropod to the bone specialist on morphology and genetics. The same resultd were found for *Cordesia* sp. nov., it is also rooted at the same clade as both former genus, and it is genetically closer from *Abyssochrysos* than from *Rubyspira*. It is the first time that molecular data was achieved for the genus *Cordesia*.

Some hypothesis can be raised with these evidences. (1) The family Provannidae is paraphyletic, (2) Provannidae should be included in Abyssochrysidae, (3) *Cordesia* (Warén and Bouchet, 2009) and now *Rubyspira* should be considered from family Abyssochrysidae, and (4) there could be more than two families within the Abyssochrysoidea. This study supports the idea that both *Rubyspira* and *Cordesia* should be considered Abyssochrysidae due to their genetic similirities, the presence of pedal furrow and similar arrangements of pallial tentacles. A morphological revision of the complete superfamily should be made because there could be more than two families in it, in that case *Cordesia* should still be classified as Abyssochrysidae, and *Rubyspira* is uncertain. However, all genera of Abyssochrysoidea are well supportes by genetic and morphology and should stay the same.

The hypothesis of organic falls as “stepping stones” for evolutionary novelties others chemosynthetic environments does not seems to apply for these group of gastropods, since in the analyzes the clade composed by the bone specialist *Rubyspira*, the bacterial mats grazer *Cordesia* and by *Abyssochrysos*, known for not inhabiting chemosynthetic habitats, were never basal. They are for certain a more derived group, that do not dwell on hydrothermal vents as the

other genus of *Abyssochrysoidea*. This study supports the idea that these more reduced environments were the “stepping stones” for this gastropods that are not found on these habitats and not the inverse.

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## SUPPLEMENTARY MATERIAL

### Appendix 1. Taxonomic and genetic descriptions

Mollusca Linnaeus, 1758;

Gastropoda Cuvier, 1797;

Caenogastropoda Cox, 1959;

Abyssochrysoidea Tomlin, 1927;

**Family not settled;**

*Remarks:* The genus of abyssochrysoid snails *Rubyspira* was not settled at any family level because no other living member of the superfamily resemble the described species. Their shell bears a remarkable resemblance to the fossil *Atresius liratus* (Gabb, 1869) from ancient cold seeps (Kiel et al., 2008; Johnson et al., 2010)

Genetic studies suggest that it is closer related to the genus *Abyssochrysos* than to other provannid. This is supported by morphological traits, such as the presence of pedal furrows, similar arrangement of pallial tentacles and similar paraspermatozoa (Johnson et al., 2010; this study).

*Rubyspira* Johnson et al., 2010;

*Remarks:* *Rubyspira* species are abyssochrysoid snails with tall shells and planktotrophic development. They present a large pallial tentacle on the right of the snout and a single small on left corner. A pedal furrow is also found. And there no traces of siphonal canal.

*Rubyspira osteovora* and *Rubyspira goffrediae* were previously described from a whale fall from Monterey Bay, California, that is the type locality (Johnson et al., 2010). *Rubyspira osteovora* is the type species for the genus, it present chemosymbionthic bacteria in their gills, but their role are unknown. Both are bone eaters and large pieces of fragments can be found on their gut content.

A third species was found on Southwest Atlantic Ocean, as was not described yet (see Sumida et al., 2016). It presents the same characteristics of the group. It is referred in the present study as *Rubyspira* sp. 3.

### ***Rubyspira* sp. nov. 1**

Material examined: ca. 880 specimens. Collected in all sites of the present study. 25°54'S, 45°01.8'W, 1508m depth (SP-1500), October 2014. 21°27'S, 39°54'W, 1491m depth (ES-1500); 22°50.4'S, 38°25.2'W, 3322m depth (ES-3300); 25°20.4'S, 39°38.4'W, 3285m depth (RJ-3300); 28°01.8'S, 43°31.8'W, 3358m depth (SP-3300), May/June 2015.

#### Description:

*Shell.* Tall and thin, with smooth spiral sculpture and axial sculpture absent. Preserved and living specimens white, without patterns of pigmentation. Aperture nearly half of shell height, teardrop shape, and siphonal channel absent. Axial and spiral sculpture are distinct on the larval shell, the protoconch has around 2.5 whorls and is sealed with a calcareous plug resembling that of the provannids, apical whorls autotomized. Transition between the teleoconch and the larval shell well-defined. Larger animals with four whorls on the teleoconch, slightly increasing in diameter. Peristome expanded, directed to the side along the aperture. No presence of marks on the collumela (Figure 2). Suture well marked. Specimens with 1.3 mm to 23.3 mm in shell height and 0.75 mm to 9.0 mm in width (Figure 1).

*Soft parts.* Cephalic tentacles without eye lobes, ventro-apical mouth, flat and large snout. Relative small head-foot. One large pallial tentacle on the right side of the snout, and a smaller one on left corner of pallial margin. Male aphyallic. The lower part of the foot is marked with a furrow (Figure 3).

*Operculum.* Thin, yellowish, distinct growth lines, paucispiral with nucleus 20-25% height, width 2/3 height of the operculum.

*Radula*. Taenioglossan, formula 2 + 1 + C + 1 + 2; ca 1.4mm long, five times as long as broad in adult specimens. Simplified central teeth, apically rounded, triangular, lateral teeth slightly taller than central ones, hook-liked, both marginal teeth are the same size and shape, with crowbar-like cuspid (Figure 4).

*Jaw*. Not seen.

*Remarks*. The occurrence of specimens in different sizes, development stages and in all sites of the present study indicate a continuous rate of recruitment, suggesting that larvae supply appears to be common in Southwest Brazil deep-sea along the year. Well distributed along all sites, in exception of SP-3300, where only 2 specimens were collected. Most of animals were collected dwelling on whale bones.

Adult specimens of *Rubyspira* sp. nov. 1 resemble *Rubyspira osteovora* in regarding the shell and radula. However, both species can be distinguished by the presence of a smoother spiral sculpture in *Rubyspira* sp. nov. 1. Juvenile specimens can be easily confused with juveniles of *Rubyspira* sp. nov. 2, but *Rubyspira* sp. nov. 1 presents a deeper suture and more ovate adult shell whorls. Traits of the larval shell, such as many number of whorls and strong axial and spiral sculpture can possibly indicate planktotrophic behavior a characteristic of the group

The intraspecific similarity of *Rubyspira* sp. nov. 1 was higher than 99% for cytochrome oxidase c subunit I gene. This kinship remains around 90% for *Rubyspira osteovora*, *Rubyspira* sp. 3 and *Rubyspira* sp. nov. 2, and 84% for *Rubyspira goffrediae*. For 16S, intraspecific similarities are smaller, and the interspecific similarities follows the same pattern (Table 1).

Distribution: Only Know from the sites of this study. Southwest Atlantic deep-sea, in organic falls from 1491 m to 3358 m depth

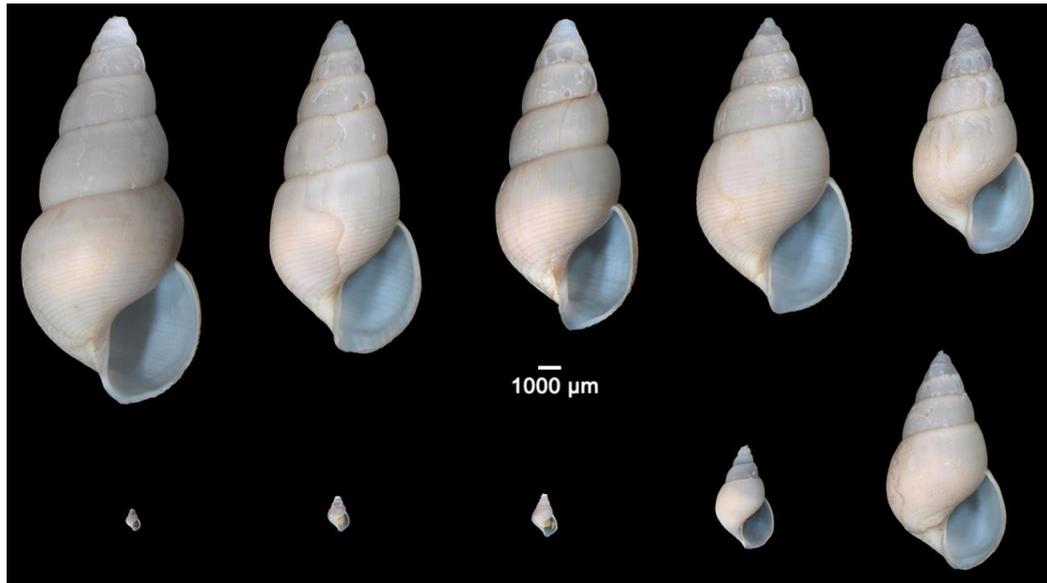


Figure 1: Plate with growth stages of *Rubyspira* sp. nov. 1. Including the largest and smallest individual, photos under stereomicroscope. The fully-grown specimens present a more elongated adult shell.

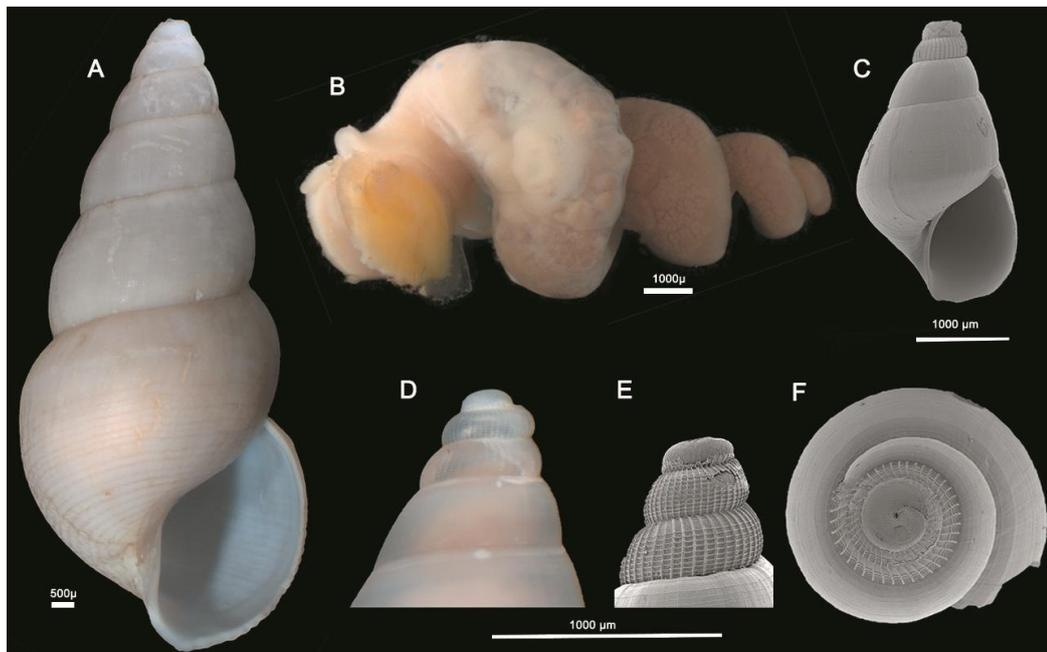


Figure 2: Stereomicroscopic photography (A, B, D) and Scanning Electron Microscopy (C, E, F) *Rubyspira* sp. nov. 1, morphology of adult shell, larval shell and soft tissue.



Figure 3: Detail of *Rubyspira* sp. nov. 1 pallial tentacle.

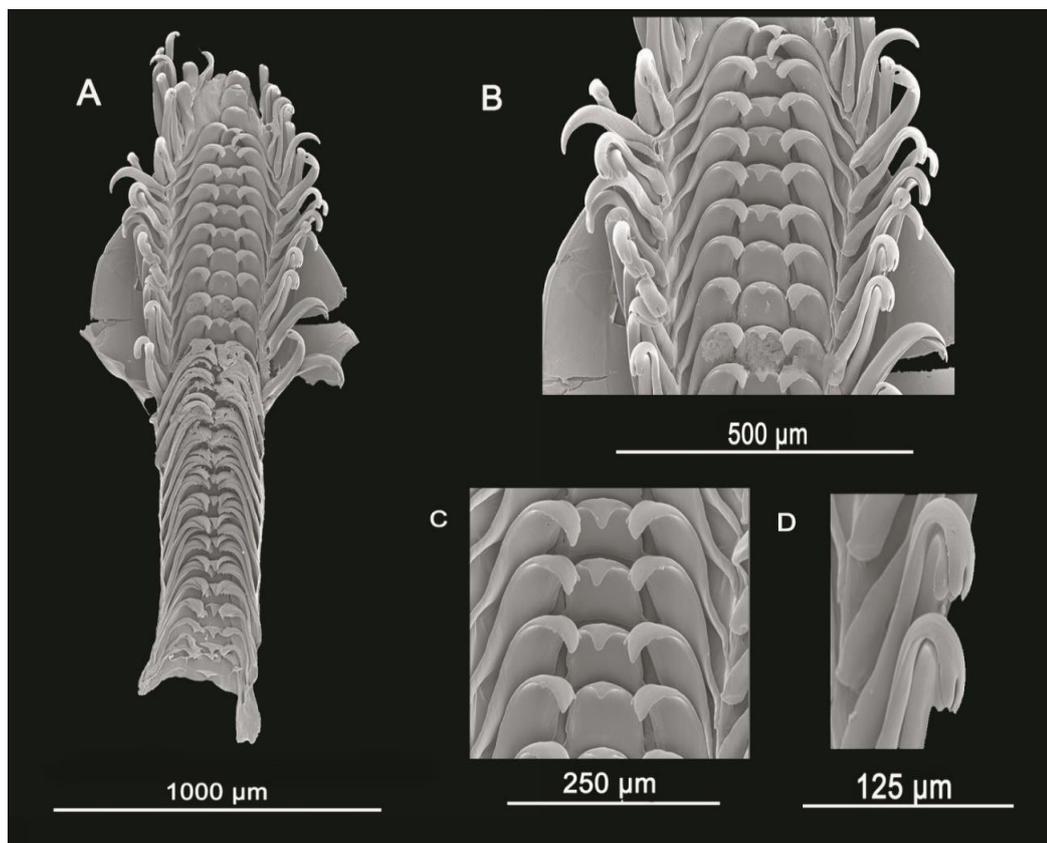


Figure 4: Scanning electron microscope of *Rubyspira* sp. nov. 1 radular teeth. A: complete radula, B: teeth details, C: Central and lateral teeth details, D: Marginal teeth detail.

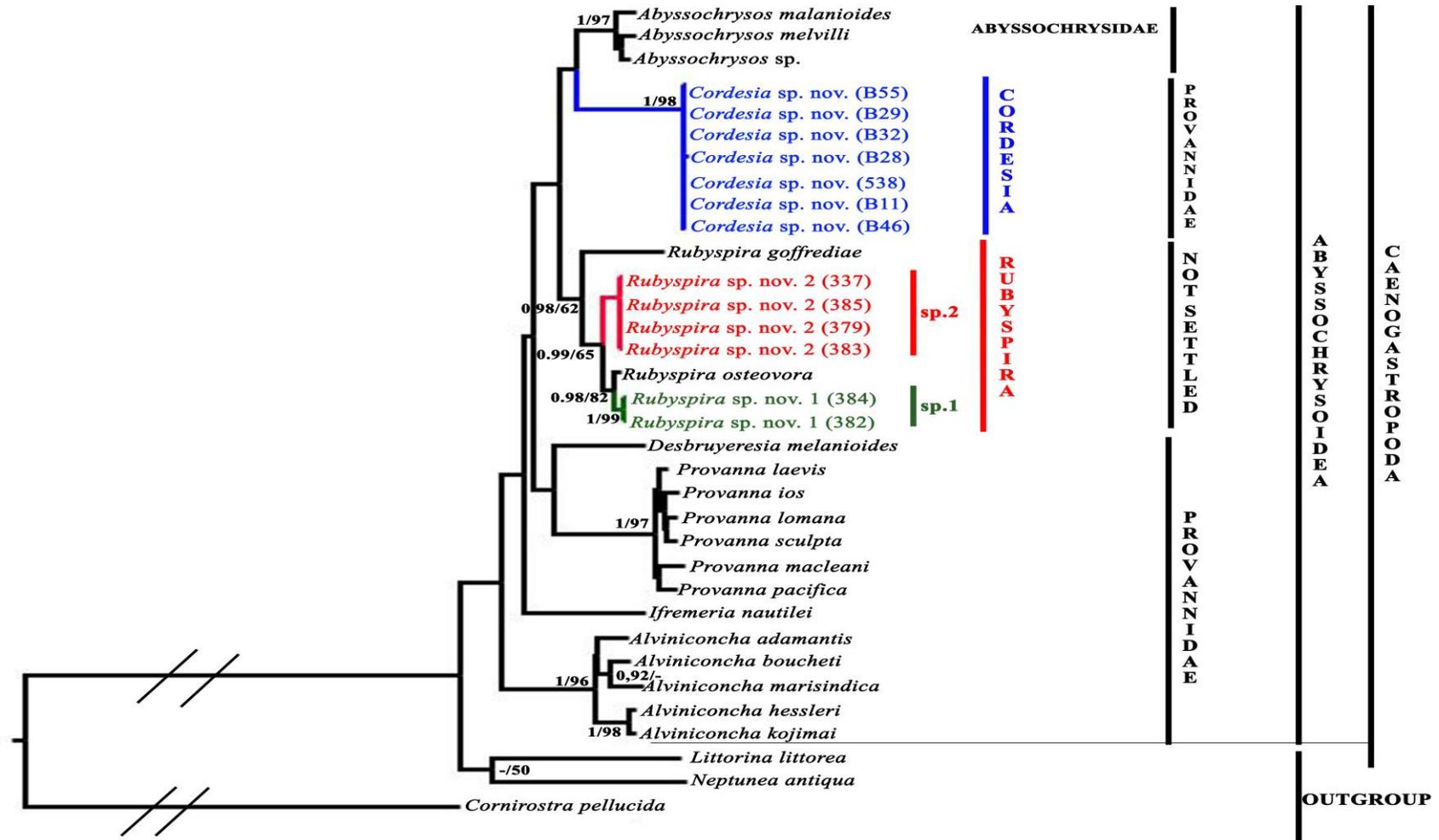


Figure 5: Phylogenetic tree of COI and 16S of Abyssochrysoide gastropods. Showing the systematic position of new species of *Cordesia* and *Rubyspira*. Both Bayesian inference and maximum likelihood (GTR + G + I) returned similar topology, nodes values shown are BI/ML. Support with less than 50% are not shown for ML. The branch with *Cornirostra pellucida* was cut for better resolution.

***Rubyspira* sp. nov. 2**

Material examined: ca. 50 specimens. Collected in 21°27'S, 39°54'W, 1491 m depth (ES-1500), October 2014.

## Description:

*Shell.* Tall shell and very smooth spiral sculpture, Turritella-like. Multispiral. Preserved and living specimens translucent white. Protoconch of the same color as adult shell, with 2-2.5 whorls, apical whorls autotomized and sealed with a calcareous plug as normal for the genus, it present axial and spiral sculptures. No siphonal canal. Limit between larval shell and teleoconch is well defined. Almost plain suture. Aperture 1.5 times as high as broad (Figure 7). Larger animals with four or five whorls on the teleoconch, slightly increasing in diameter. Collumela is not marked. Specimens with 2.9 mm to 10.7 mm in shell height and 1.3 mm to 4.8 mm in width (Figure 6).

*Soft parts.* Ventro-apical mounth, flat and small snout. Cephalic tentacles without eye lobes. Males aphalic. One large pallial tentacle on the right, and a smaller one on left corner of pallial margin.

*Operculum.* Thin. Yellowish and transparent. Paucispiral with nucleus 35-40% height, windth 2/3 height.

*Radula.* Taenioglossan, formula 2 + 1 + C + 1 + 2; 5.5 times as long as broad in large specimens. Central, lateral and marginal sturdy teeth have same shape and size, excepted by the central teeth that are smaller and wider, finger-like structures on cuspids that increases in number from the middle to periphery of its dentition (Figure 8).

*Jaw.* Not seen.

*Remarks.* Only few supposed fully grown specimens of *Rubyspira* sp. nov. 2 were found, most specimens collected were juveniles. All animals were at the same site of the present study and were found on whale bones and wood parcels.

*Rubyspira* sp. nov. 2 has a slender shell than *Rubyspira* sp. nov. 1, and its suture is much smoother, however they can be easily mixed at early stages of development. Its radula differs a lot from other snails of the genus.

Similarities of *Rubyspira* sp. nov. 2 specimens were higher than 99% for cytochrome oxidase c subunit I gene. This kinship remains around 90% for *Rubyspira osteovora*, *Rubyspira* sp. nov. 1 and *Rubyspira* sp. 3, and 84% for *Rubyspira goffrediae*. For 16S, intraspecific similarities are smaller, however are higher considering other species of the genus (Table 1).

Distribution: Only Know from the sites of this study. Southwest Atlantic deep-sea, in organic falls at 1491 m depth.

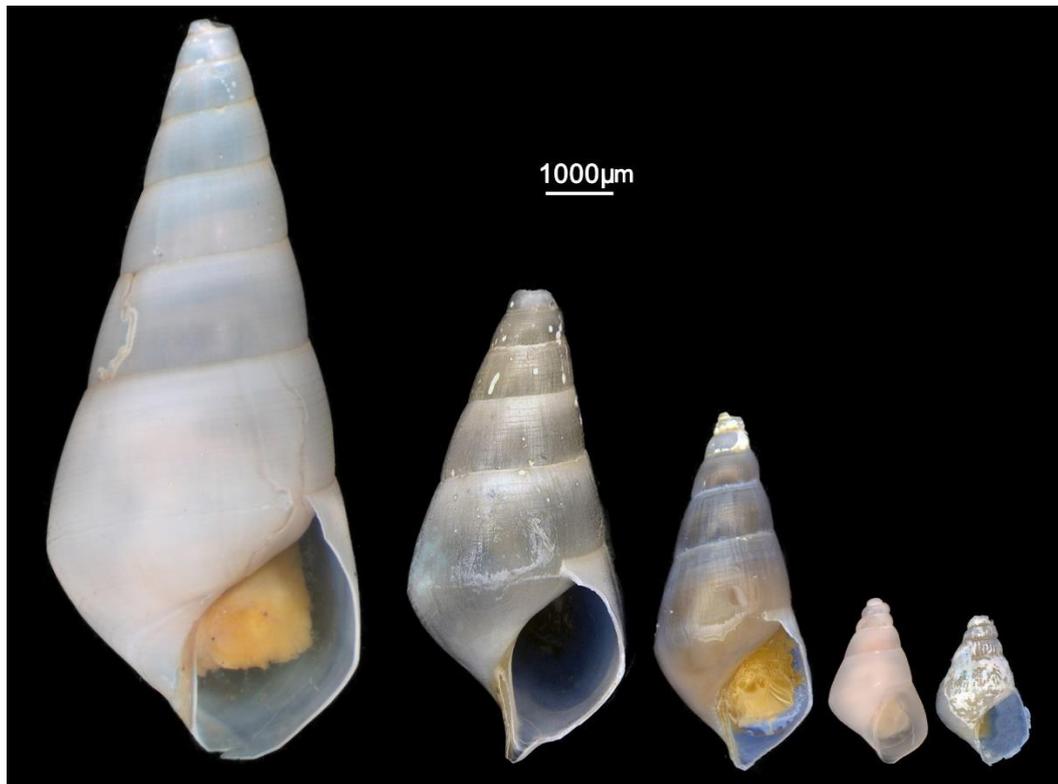


Figure 6: Plate with growth stages of *Rubyspira* sp. nov. 2 including the largest and smallest individual, photos under stereomicroscope. The fully-grown specimens present a more elongated adult shell.

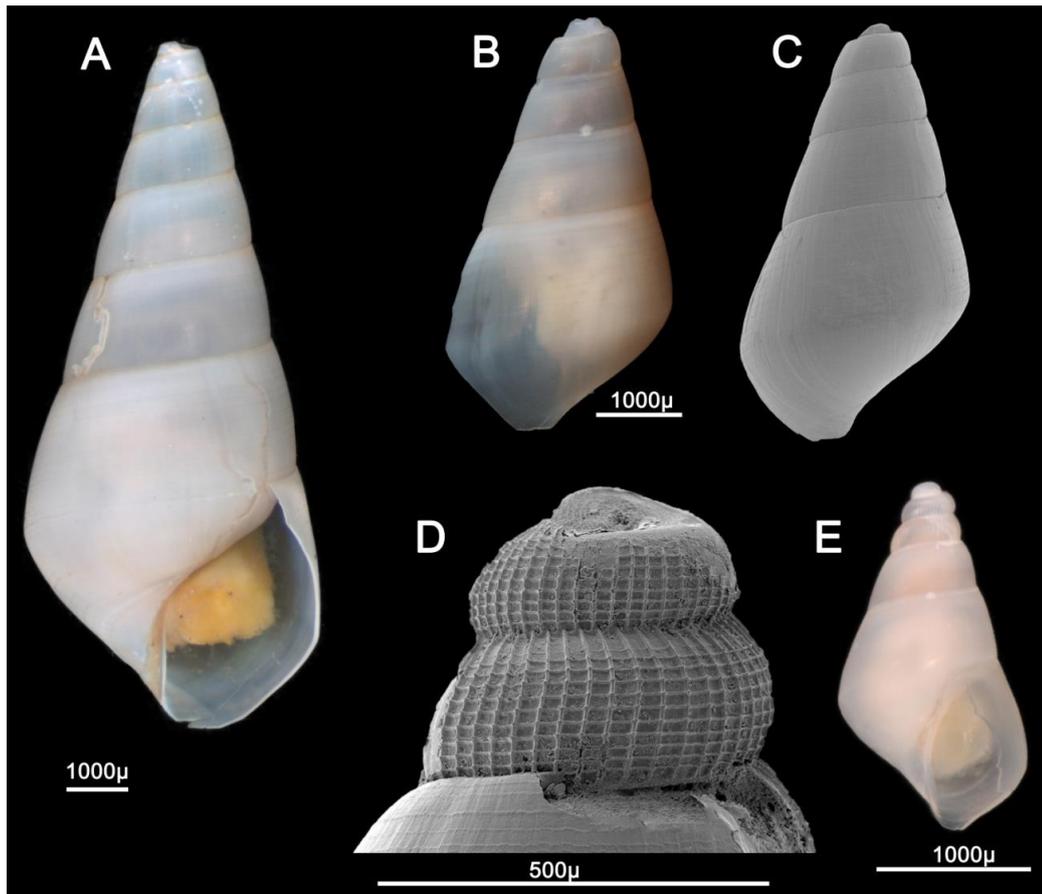


Figure 7: Stereomicroscope photography (A, B, E) and Scanning Electron Microscopy (C, D) of *Rubyspira* sp. nov. 02, showing the morphology of adult shell and larval shells.

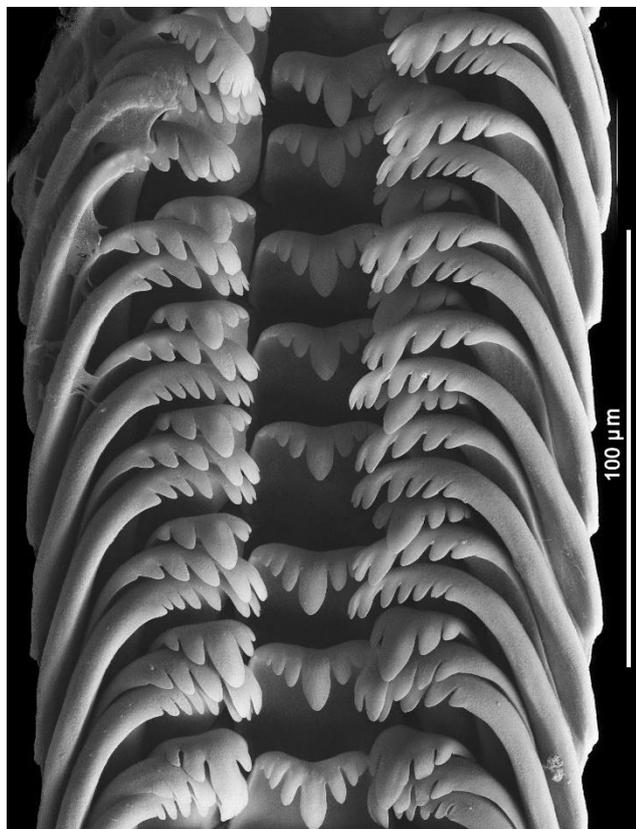


Figure 8: Scanning electron microscopy of the complete dentition of *Rubyspira* sp. nov. 2. radula.

**Provannidae** Warén and Ponder, 1991;

*Remarks:* Provannid snails are known from chemosynthetic environments, such as hydrothermal vents, cold seeps and organic falls (Bouchet and Warén, 1991; Warén and Ponder, 1991; Warén and Bouchet, 1993; Warén and Bouchet, 2009; Johnson et al., 2015; Chen et al., 2016a, 2016b).

Many species are described for this family, and it is formed by 5 genera, *Provanna* (Dall, 1918), *Alviniconcha* (Okutani and Ohta, 1988), *Ifremeria* (Bouchet and Warén, 1991), *Desbruyeresia* (Warén and Bouchet, 1993), and *Cordesia* (Warén and Bouchet, 2009).

***Cordesia*** Warén and Bouchet, 2009;

*Remarks:* The genus is characterized by males with cylindrical penis, differing then from other provannid snails. Two large palial tentacles on the right, lower one much smaller.

Warén and Bouchet (2009) suggested that the genus *Cordesia* should be classified as Provannidae and that the penis was a plesiomorphic trait and could be lost on other gastropods of the group. However, the presence of both pallial tentacles could only be compared to *Abyssochrysos*. Opening the question if it really should be classified as Provannidae or Abyssochrysidae, or if the Provannidae should be included on the other family.

In the present study is suggested that *Cordesia* should be classified as Abyssocrysidae due to morphological and genetic similarities to *Abyssochrysos*. It was the first time that genetic analyses were conducted for this group.

***Cordesia* sp. nov.**

Material examined: ca. 380 specimens. Collected in 22°50.4'S, 38°25.2'W, 3322m depth (ES-3300); 25°20.4'S, 39°38.4'W, 3285m depth (RJ-3300); 28°01.8'S, 43°31.8'W, 3358m depth (SP-3300), May/June 2015.

## Description:

*Shell.* Normal size for its family, short and broad with very weak axial sculpture (lines) and well defined spiral sculpture, some individual has a curling on the

adult whorl. Preserved specimens brownish. Periostracum forms small bristles. Large aperture, about 60% of shell height, with a distinct but small siphonal channel. Larval shell consisting of 2 whorls, with axial and spiral sculpture well defined and visible, the initial part is slightly opened and sealed with a calcareous structure. The limit between the teleoconch and protoconch is well-marked. Teleoconch with more than 3 whorls on bigger animals, increasing rapidly in diameter, connected by a visible suture. Peristome expanded on the right lower part (Figure 10). Columella marked by a small furrow curved to the right and forward on its bottom part. Specimens with 1.6 mm to 7.2 mm in shell height and 1.1 mm to 4.8 mm in width (Figure 9).

*Soft parts.* Males possess a cylindrical penis, two right pallial tentacles, one of them much smaller than the other. Head-foot of normal size for the group, with a large snout. Cylindrical cephalic tentacles without eye-lobes.

*Operculum.* Yellowish, very thin for the smaller individual and goes thicker with size. Growth lines paucispiral and distinct, nucleus at 25% of height. Width 2/3 of height.

*Radula.* Taenioglossan, formula  $2 + 1 + C + 1 + 2$ ; ca 1.8 mm long, narrow, twelve times longer than broader on adult specimens. Strong marginal teeth, apically denticles, longer and less abundant on inner ones. Lateral teeth with apical cusp, one strong central denticle, one or two lateral ones, followed by a finger like denticle on teeth lateral. Central tooth with expanded base, triangular-shape, with apical denticle (Figure 11).

*Jaw.* Not seen.

*Remarks.* Most of the individuals were collected on the control substrate, but they were also abundant on the woods, and less present on the whale bones. All specimens were collected at 3300 sites of the present study. It was the first time *Cordesia* were found on organic falls.

The presence of gastropods of different sizes and stages of development suggests indicates recruitment on Southwest Atlantic deep-sea along the year.

Many numbers of whorls, axial and spiral sculptures on the larval shell indicates a planktotrophic development.

Intraspecific similarities are higher than 97.5% for COI gene, and even higher for 16S. For both genes and for *Cordesia* sp. nov. and both new *Rubyspira* species, the genus *Abyssochrysos* appears to be less distant than other provannid snails (Table 1).

Distribution: Only Know from the sites of this study. Sothwest Atlantic deep-sea, in organic falls from 3285 m to 3358 m.

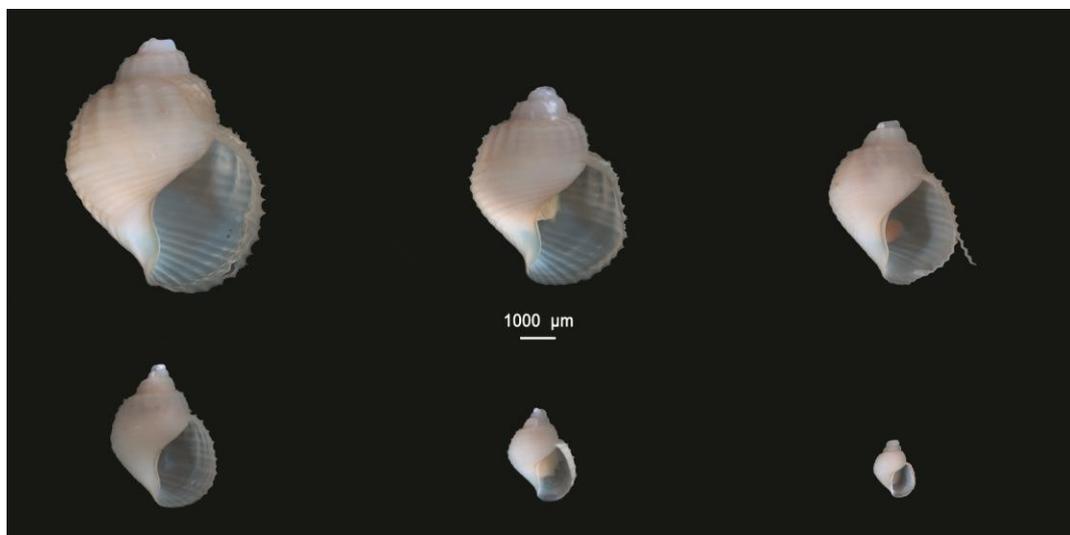


Figure 9: Plate with stages of development of *Cordesia* sp. nov., including the largest and the smallest individual found, photos under stereomicroscope. Juveniles and fully-grown specimens have similar shell shape.

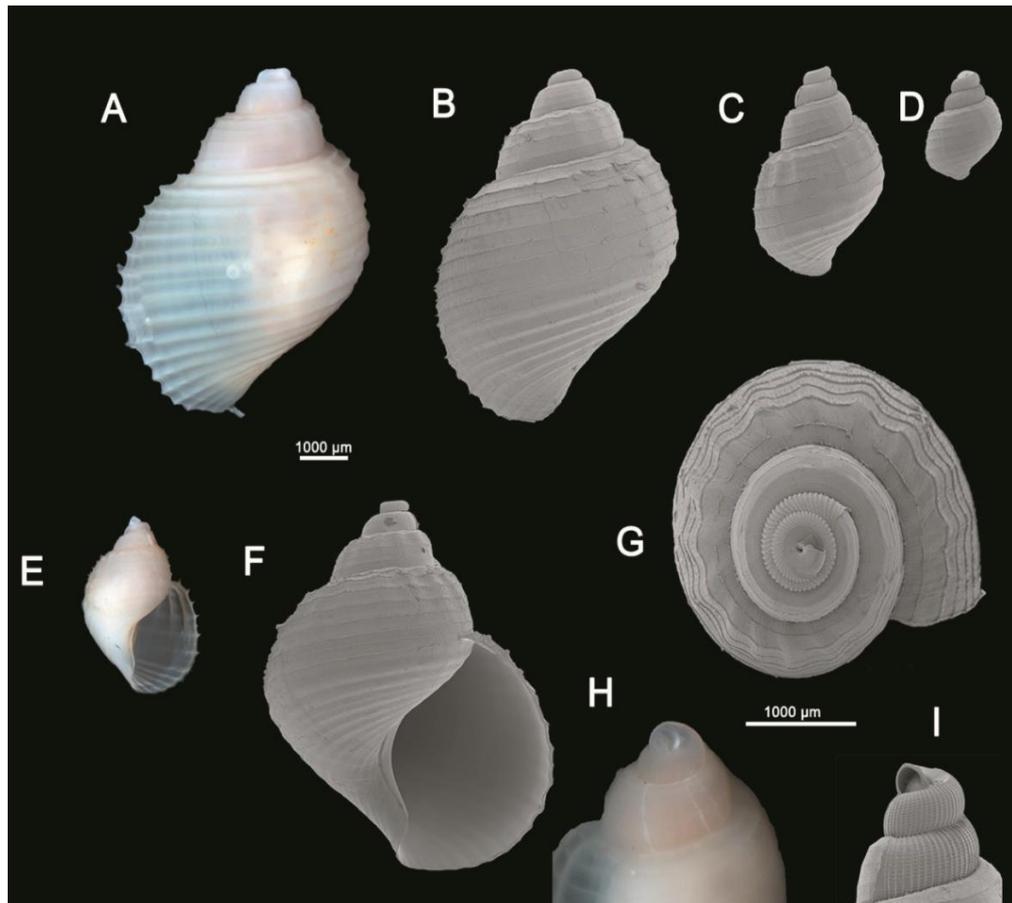


Figure 10: *Cordesia* sp. nov. plate. Scanning electron microscope (B, C, D, F, G, I). Photos under stereo microscope (A, E, H). Adult shell (A, B, C, D, E, F, G). Protoconch detail (H, I).

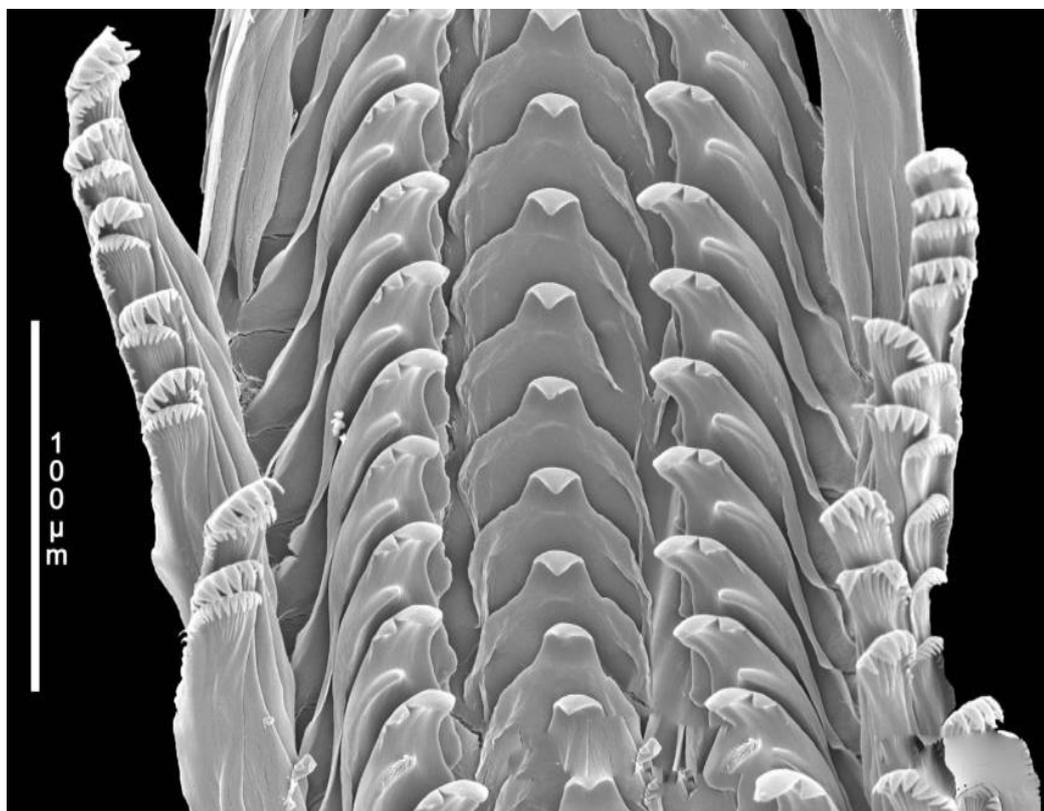


Figure 11: Scanning electron microscope of *Cordesia* sp. nov. radular teeth.

Table 1: DNA sequence similarity identity (lower left triangle) for mtCOI among genera of Abyssochrysoidea and outgroups, *Rubyspira* sp. nov. 1, *Rubyspira* sp. nov. 2 and *Cordesia* sp. nov. are at species level. (-) spaces are equal to 100% due to unique specimen.

	<i>Rubyspira</i> sp. nov. 1	<i>Rubyspira</i> sp. nov. 2	<i>Rubyspira</i>	<i>Cordesia</i> sp. nov.	<i>Alviniconch</i> <i>a</i>	<i>Ifremeria</i>	<i>Provanna</i>	<i>Desbruyeresia</i>	<i>Abyssochryso</i> <i>s</i>	<i>Littorina</i>	<i>Neptunea</i>	<i>Cornirostr</i> <i>a</i>	N
<i>Rubyspira</i> sp. nov. 1	99.32												3
<i>Rubyspira</i> sp. nov. 2	92.29	99.78											4
<i>Rubyspira</i>	92.03	91.84	92.09										10
<i>Cordesia</i> sp. nov.	84.20	84.62	84.18	99.77									9
<i>Alviniconcha</i>	83.59	83.76	83.56	82.93	88.84								6
<i>Ifremeria</i>	81.10	80.31	80.47	83.00	82.60	-							1
<i>Provanna</i>	82.10	83.15	82.28	82.84	81.62	81.62	92.34						6
<i>Desbruyeresia</i>	84.62	85.34	84.25	82.24	82.49	80.31	82.82	87.31					3
<i>Abyssochryso</i> <i>s</i>	85.56	85.78	85.56	86.53	84.36	81.40	84.32	84.90	92.12				3
<i>Littorina</i>	79.95	80.96	80.09	80.84	76.59	78.56	82.19	78.12	80.74	-			1
<i>Neptunea</i>	80.88	81.34	81.03	82.06	79.54	78.56	81.29	79.43	82.06	82.06	-		1
<i>Cornirostra</i>	71.87	72.43	72.10	71.08	73.09	71.55	73.31	73.09	72.65	71.77	73.30	-	1

**Appendix 2.** Relative abundance of the main gastropod species collected on the landers implanted during the BioSuOr Project on each locality and latitude on the continental margin off Southeast Brazil. The total abundance of these species is 97%, been one reason why they were selected to be analysed. Percentages are related to total gastropod fauna.

Species		ES			RJ		SP			Total
		1500	3300	Total	3300	Total	1500	3300	Total	
<i>Hyalogyrina rissoela</i>	n	939	77	<b>1016</b>	91	<b>91</b>	11	2657	<b>2668</b>	<b>3775</b>
	% total	24.9%	2.0%	<b>26.9%</b>	2.4%	<b>2.4%</b>	0.3%	70.4%	<b>70.7%</b>	
	% lander	70.5%	26.4%	<b>62.6%</b>	11.8%	<b>11.8%</b>	4.8%	92.6%	<b>86.1%</b>	
<i>Rubyspira</i> sp. nov. 1	n	267	79	<b>346</b>	356	<b>356</b>	182	4	<b>186</b>	<b>888</b>
	% total	30.1%	8.9%	<b>39.0%</b>	40.1%	<b>40.1%</b>	20.5%	0.5%	<b>20.9%</b>	
	% lander	20.0%	27.1%	<b>21.3%</b>	46.1%	<b>46.1%</b>	80.2%	0.1%	<b>6.0%</b>	
<i>Rubyspira</i> sp. nov. 2	n	53	0	<b>53</b>	0	<b>0</b>	0	0	<b>0</b>	<b>53</b>
	% total	100.0%	0.0%	<b>100.0%</b>	0.0%	<b>0.0%</b>	0.0%	0.0%	<b>0.0%</b>	
	% lander	4.0%	0.0%	<b>3.3%</b>	0.0%	<b>0.0%</b>	0.0%	0.0%	<b>0.0%</b>	
<i>Cordesia</i> sp nov.	n	0	106	<b>106</b>	236	<b>236</b>	0	34	<b>34</b>	<b>376</b>
	% total	0.0%	28.2%	<b>28.2%</b>	62.8%	<b>62.8%</b>	0.0%	9.0%	<b>9.0%</b>	
	% lander	0.0%	36.3%	<b>6.5%</b>	30.6%	<b>30.6%</b>	0.0%	1.2%	<b>1.1%</b>	
<i>Lusitanops cingulatus</i>	n	15	22	<b>37</b>	69	<b>69</b>	1	128	<b>129</b>	<b>235</b>
	% total	6.4%	9.4%	<b>15.7%</b>	29.4%	<b>29.4%</b>	0.4%	54.5%	<b>54.9%</b>	
	% lander	1.1%	7.5%	<b>2.3%</b>	8.9%	<b>8.9%</b>	0.4%	4.5%	<b>4.2%</b>	
<b>TOTAL</b>	n	1274	284	<b>1558</b>	752	<b>752</b>	194	2823	<b>3017</b>	<b>5327</b>
	% total	23.2%	5.2%	<b>28.4%</b>	13.7%	<b>13.7%</b>	3.5%	51.4%	<b>54.9%</b>	
	% lander	95.6%	97.3%	<b>95.9%</b>	97.4%	<b>97.4%</b>	85.5%	98.4%	<b>97.4%</b>	

**Appendix 3.** Number of gastropod individuals per species sampled on the different substrates collected in the Southeast Brazilian continental margin during the BioSuOr Project and their distribution on substrate and depths.

Species	Control			% species / substrate	Whale Bone			% species / substrate	Wood			% species / substrate	Total
	1500	3300	Total		1500	3300	Total		1500	3300	Total		
<i>Pseudococculinidae</i> sp.	0	0	0	0.0%	0	0	0	0.0%	1	1	2	100.0%	2
<i>Hyalogyrina rissoella</i>	0	163	163	4.3%	950	2645	3595	95.2%	0	17	17	0.5%	3775
<i>Abyssochryso</i> s cf. <i>melanioides</i>	2	0	2	4.7%	40	0	40	93.0%	0	1	1	2.3%	43
<i>Rubyspira</i> sp. nov 1	11	3	14	1.6%	437	359	796	89.6%	1	77	78	8.8%	888
<i>Rubyspira</i> sp. nov 2	1	0	1	1.9%	26	0	26	49.1%	26	0	26	49.1%	53
<i>Lusitanops cingulatus</i>	1	50	51	21.7%	6	130	136	57.9%	9	39	48	20.4%	235
<i>Cordesia</i> sp. nov	0	164	164	43.6%	0	64	64	17.0%	0	148	148	39.4%	376
<i>Valvatoidea</i> sp. 1	4	21	25	30.5%	4	1	5	6.1%	38	14	52	63.4%	82
<i>Raphitomidae</i> sp. 2	0	0	0	0.0%	0	1	1	50.0%	0	1	1	50.0%	2
<i>Morphotype 1</i>	0	0	0	0.0%	0	0	0	0.0%	0	1	1	100.0%	1
<i>Eulimidae</i> sp.	0	0	0	0.0%	1	0	1	100.0%	0	0	0	0.0%	1
<i>Raphitomidae</i> sp. 3	0	0	0	0.0%	0	4	4	80.0%	1	0	1	20.0%	5
<i>Valvatoidea</i> sp. 2	0	14	14	66.7%	0	7	7	0.0%	0	0	0	0.0%	21
<i>Rubyspira</i> sp. 3	0	0	0	0.0%	0	9	9	100.0%	0	0	0	0.0%	9
<b>% substrate-depth / total</b>	<b>0.3%</b>	<b>7.6%</b>			<b>26.7%</b>	<b>58.6%</b>			<b>1.4%</b>	<b>5.4%</b>			<b>100.0%</b>
<b>Total</b>	<b>19</b>	<b>415</b>	<b>434</b>	<b>7.9%</b>	<b>1464</b>	<b>3220</b>	<b>4684</b>	<b>85.3%</b>	<b>76</b>	<b>299</b>	<b>375</b>	<b>6.8%</b>	<b>5493</b>

**Appendix 4.** Growth plate of *Hyalogyrina risseoela*. Including the largest and smallest individual, photos under stereomicroscope. Juveniles and adult specimens have similar shell shape. Holotype and paratypes (undefined).



**Appendix 5.** Growth plate of *Lusitanops cingulatus*. Including the largest and smallest individual, photos under stereomicroscope. Adult specimens loses the brownish larval shell. Holotype and paratypes (undefined).

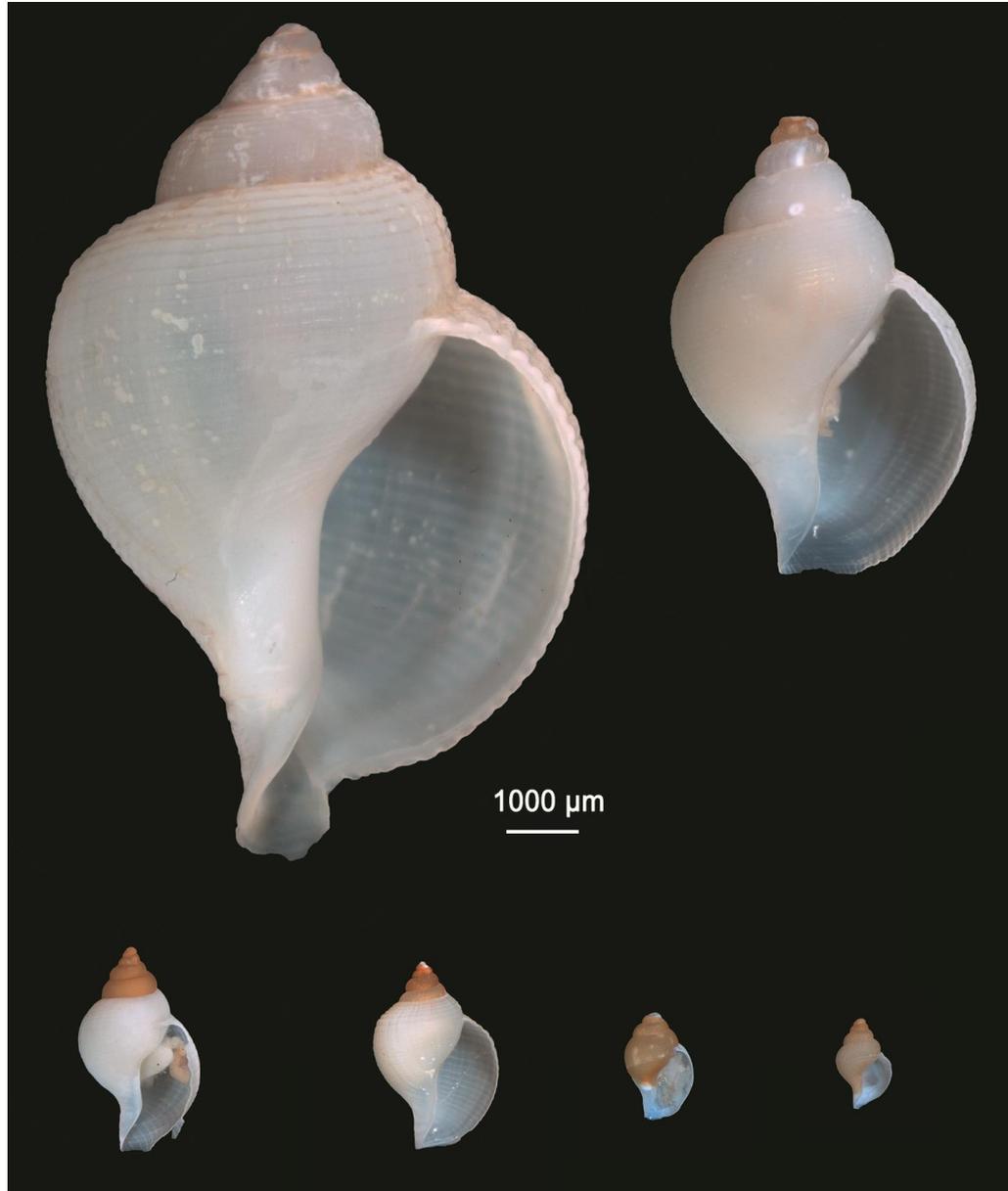


Figure 2.14: Plate with growth stages of *Lusitanopscingulatus*, including the largest and smallest individual, photos under stereomicroscope. The gradual loss of larval shell is common on larger specimens.

**Appendix 6.** Table 2.3. DNA sequence similarity identity (lower left triangle) for mtCOI and among Heterobranchia genera. *Hyalogyrina* is represented only by *Hyalogyrina rissoela*.

	<i>Hyalogyrina</i>	<i>Valvata</i>	<i>Cornirostra</i>	<i>Architectonica</i>	<i>Omalogyra</i>	<i>Ebala</i>	<i>Cima</i>	<i>Orbitestella</i>	<i>Pupa</i>	<i>Rissoella</i>	<i>Tomthompsonia</i>	<i>Littorina</i>	<i>N</i>
<i>Hyalogyrina</i>	99.73												5
<i>Valvata</i>	80.44	89.42											2
<i>Cornirostra</i>	73.49	76.46	-										1
<i>Architectonica</i>	59.78	60.59	60.95	-									1
<i>Omalogyra</i>	62.34	63.14	59.49	67.88	-								1
<i>Ebala</i>	73.59	75.82	71.17	59.85	60.40	-							1
<i>Cima</i>	73.10	74.05	70.92	57.34	61.41	74.73	-						1
<i>Orbitestella</i>	75.14	77.47	72.26	59.31	63.87	72.45	75.54	-					1
<i>Pupa</i>	68.31	67.52	64.78	53.65	57.85	66.42	74.46	68.25	-				1
<i>Rissoella</i>	67.29	70.72	65.61	57.12	60.86	70.63	76.77	70.53	69.07	78.17			2
<i>Tomthompsonia</i>	72.94	74.54	70.07	60.22	62.59	73.54	83.15	73.72	73.91	73.36	-		1
<i>Littorina</i>	74.59	75.41	72.47	60.73	61.13	73.48	70.14	74.90	69.23	68.93	71.46	-	1

**Appendix 7.** Phylogenetic tree of COI and 16S of heterobranch gastropods Showing the systematic position of *Hyalogyrina rissoela*. Both Bayesian inference and maximum likelihood (GTR + G + I) returned the same topology, nodes values shown are BI/ML. Support with less than 50% are not shown for ML.

